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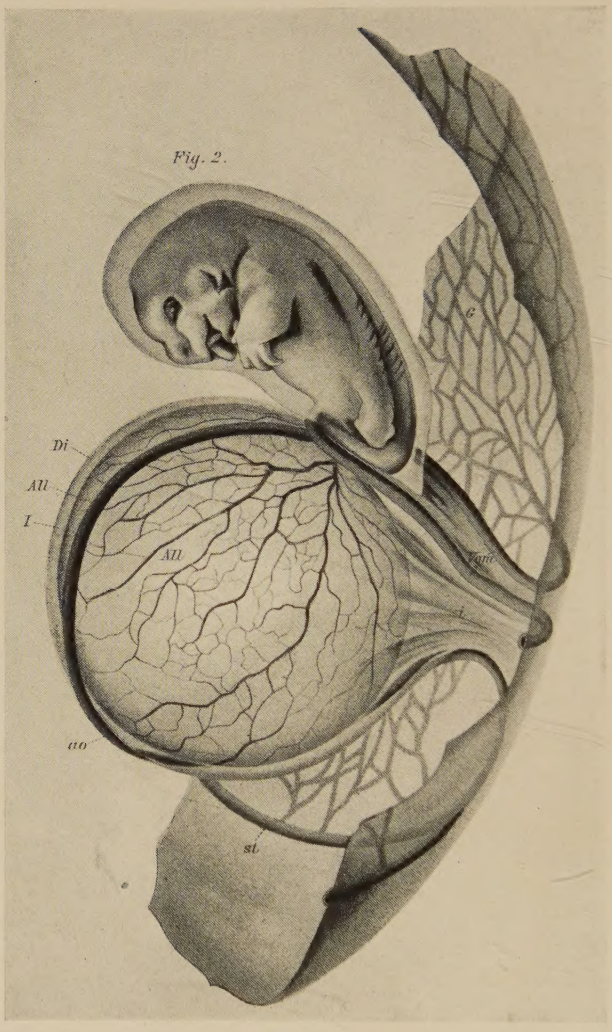

Opossum embryo of the twelfth day showing chorion, amnion allantois and body stalk. Taken from Selenka, 1887.

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THE EMBRYOLOGY OF THE OPOSSUM

EDWARD McCRADY, JR.

The Effingham B. Morris Biological Farm of The Wistar Institute

SIXTY-SIX TEXT FIGURES AND THREE PLATES

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To begin with, I cannot be too enthusiastic in expressing my indebtedness to Dr. Carl Gottfried Hartman. His studies on the opossum have been more extensive than those of any previous investigator. That they have been liberally drawn upon in the following pages will be apparent from the frequent appearance of his name in the text. Not only has my account of cleavage, germ-layer formation, and the rate of development, been based primarily upon his researches, but even my studies of the later stages which he did not examine microscopically, have been made, for the most part, upon material which he collected and left at The Wistar Institute.

I have come to differ with Doctor Hartman's interpretations in several respects which are discussed in the appropriate parts of the text; but these differences, which, incidentally, do not involve any differences in actual observations within the stages he studied, came to light only after I had had the advantage of his fundamental researches and the opportunity to study a large series of later stages of which he had no sections, and which threw new light on the significance of the earlier material.

To Dr. C. H. Heuser, also formerly of The Wistar Institute, I am indebted for the negatives of most of the photographs of external views of the embryos, and for the use of figure 60. In the few cases in which I had to make the negatives of external views myself, the difficulties I encountered impressed me very strongly with my indebtedness to his exceptional skill.

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INTRODUCTION

The Virginia opossum (Didelphys virginiana Kerr) [1] is very abundant and easily caught in almost every part of the United States from about the latitude of Pennsylvania south. Being a marsupial, and an extremely primitive one, it would have been studied very thoroughly and used very widely for laboratory purposes long ago, if it had been more amenable to domestication.

The first serious attempt to breed this animal in captivity was that of Dr. Emil Selenka, who imported a large number of them to Erlangen, Germany, some time just prior to 1887. He had the good fortune to obtain several litters of eggs and embryos before the colony went sterile and the animals died. He tried the same experiment also with the South American opossum, Didelphys cancrivorous, and with three Australian marsupials (Hypsiprymnus penicillatus, Phalangista vulpina, and Phalangista orientalis); but in these ventures he met with less success than he had had with the Virginia opossum.

Since his time several American zoölogists and embryologists have tried to establish breeding colonies of Didelphys, and all have met with failure until The Wistar Institute in 1930 decided to invest a considerable sum of money in a large-scale effort to solve the problem. After several years of disappointment the major difficulties seem at last to have been overcome, and for the convenience of those who may want to breed the opossum in their own laboratories I shall pass on what information we have gained.

The first essential is to prevent the animals from developing rickets. Though from the very inception of our colony all the

[1] Another spelling of the generic name often seen substitutes an 'i' for the 'y' in the last syllable. Etymologically this change makes the term mean 'two dolphins or porpoises.' I prefer the spelling which means 'two uteri.'

animals were given whole milk and eggs every day, they, nonetheless, developed acute rickets. The ones which were adult when brought into the colony occasionally bred once before going sterile. They rarely lived more than a year, and finally died in tetanic convulsions similar to those often associated with osteomalacia and rickets. The young born in the colony appeared normal during the first 80 days (i.e., the nursing period), but then rapidly developed some very characteristic abnormalities. Their legs grew less rapidly than their heads and trunks, and became disproportionately short and bowed. Their heads appeared disproportionately large, and their backs showed kyphosis. Their fur was scrawny, and their teeth abnormal, the canines frequently breaking off. When the thorax was opened, the ends of the ribs showed a typical rachitic rosary. Such animals never attained sexual maturity, and rarely lived as long as a year; though I have on record one case of a male which showed all the rachitic symptoms in an extreme degree, but which survived for 2 years and 3 months.

At first I found it difficult to believe that the animals could really have rickets when they were being fed eggs and milk every day, but as soon as I saw that there was no doubt about the fact that they actually did, I thought the solution of that difficulty would be easy—we would simply add cod liver oil to the diet. This was done for 1 year without noticeable, beneficial result. Not only were the diseased specimens not cured, but normal young introduced into the colony from outside became just as abnormal with cod liver oil as without.

After trying other vitamin D concentrates, and combinations of vitamin D and vitamin A, all to no avail, the next resource was to try forcing the animals to live in the open sunlight. This was decidedly contrary to their natural inclinations, as under normal conditions they are nocturnal. But even the enforced exposure to sunlight did them no good.

Finally, I began opening the stomachs of animals just captured, to discover what the wilds ones were eating which the captive ones were not. One such thing which I noticed was

bones. Under natural conditions they eat, among other things, small vertebrates (frogs, lizards, salamanders, small birds, etc.) entire—bones and all. In our colony they were getting plenty of meat (beef, beef heart, and liver) but no bones. So after that we sprinkled bone meal into the food pans every day, and that cured the rickets. This suggests a curious calcium and phosphorus metabolism which might be worthy of further study. As their parathyroid glands are also peculiar, there may be an interesting correlation here.

After the addition of bone meal to the diet the animals grew to full, normal adult stature and proportions, and did not die prematurely; but still they did not breed. Examination of the testes of the males raised in the colony showed plenty of normal-looking, mature spermatozoa; but the ovaries of the females were definitely atrophic, and contained few or no mature graafian follicles. Hartman ('23) had reported some evidence that this condition of the ovary might be associated with inadequate opportunity for exercise, so we tried enlarging the pens and introducing devices for climbing. The nest boxes in which the animals sleep during the day were hung on racks on the walls of the colony rooms, and the food pans were placed on the floor. The animals had to climb down to feed during the night, and with the return of daylight, if they wanted a dark hole in which to hide, they had to climb back up. This they invariably did, rather than sleep on the floor in open daylight, so a certain amount of exercise was forced upon them, but they took advantage of their opportunities to a greater extent than that, and at night I often found them chasing each other up and down the tree branches which led to the nest boxes.

After that change the females began to ovulate, and we got our first litters from animals which had been raised in captivity. In the spring and summer of 1935 we had 250 young born in the colony. This year (1937) we have some third-generation, captive-born young; so we feel that at least our major problems must have been solved.

My first recommendations, therefore, for the breeding of opossums are that the breeding pens should have at least 50 square feet of floor space, and that the daily diet should include bone meal. The principal foods are meat, milk, and eggs. Vegetables and fruits are eaten, but less avidly. Wild opossums also eat beetles, grubs, and viscera, but apparently these are not necessary constituents of their diet.

I. PRELIMINARY DATA

The ovarian egg. The testicular sperm. Mating

The ovarian egg. Aside from the exceptional frequency of polyovular follicles and polynuclear ova (Hartman, '36), the general histology of the ovary of the opossum is not significantly different from that of other mammals. Certainly most, and perhaps all, of these atypical ova and follicles degenerate within the ovary, and so a detailed discussion of them is not necessary in connection with the normal embryology of the opossum.

Just before ovulation the normal egg (Hartman, '16, '19) is slightly elliptical, the average of the longest and shortest diameters being 0.165 mm. and 0.135 mm., respectively (fig. 1). This is larger than the tubal ovum. The cytoplasm includes a considerable quantity of yolk in the form of granules and fat droplets. These are not concentrated at either pole of the egg, but are principally confined to a rather diffuse spherical layer a little beneath the surface of the ovum (fig. 1B, y.g.). The cytoplasm both medial and superficial to this layer is more homogeneous.

The first maturation division (fig. 1, A) takes place within the ovary. The spindle forms in the superficial cytoplasm usually near one of the ends of the slightly elongate oocyte. The haploid number of chromosomes is eleven, all of which are tetrads in the metaphase of this division. One of these tetrads represents the synapsed X-chromosomes. The first polar body (fig. 1 B, p.b.) is spindle shaped and almost devoid of cytoplasm. It is variable in size, but averages about 0.025 mm. in its longest diameter. A distinct zona pellucida,

ABBREVIATIONS USED IN ALL FIGURES

a., atrium of heart
a.c., anterior semicircular canal
a.c.a., anterior cerebral artery
a.c.v., anterior cardinal vein
a.f., axial filament
a.g., anal musk gland
a.g.o., anal gland orifice
alb., albumen
all., allantois
a.l.r., anterior limb ridge
am.c., amniotic cavity
amp., ampulla
an., anus
ao., aorta
a.r., anterior ridge of otocyst
at., atrium of otocyst
b., bladder
b.a., branchial arch
b.p., branchial pouch
b.r., branchial ridge
c., coelom
c.a., conus arteriosus
can.r., canalis reuniens
c.c., crus commune
ce., centriole
cho., chondriosome
chr., chromosome
cl., glans clitoridis
co., cochlea
c.o., cumulus oöphorus
c.r., centriolar ring
c.t., caudal tube
c.u., cervix uteri
d.a., dorsal aorta
d.m., dorsal mesocardium
d.m.l., dorsal mesocardial line
e.d., endolymphatic duct
end., endoderm
e.s., endolymphatic sac
ex., excrescence from distal centriole
F., fallopian tube
f.g., fat globule
f.l., follicular liquid
fo., foregut
g., ganglion
h., hypophysis
H.n., Hensen's node
i., infundibulum
id., idiozome
j., jugular vein
k., metanephros
l.b., lung bud
l.c., lateral semicircular canal
l.c.v., lateral cephalic vein
l.g., lateral groove of pharynx
l.p., lung pouch
l.r., lateral ridge of otocyst
l.v.c., lateral vaginal canal
m., mesonephros .
m.c.v., middle cephalic vein
M.d., müllerian duct
met., metencephalon
m.g., medullary groove
m.p., medullary plate
m.t., mesonephric tubule
m.v.c., median vaginal cul-de-sac
n., notochord
n.c., neural crest cells
n.p., notochordal plate
nu., nucleus
n.v.ch., non-vascular chorion
o., ovum
o.a., ophthalmic artery
o.p., otic placode
op.c., optic cup
op.l., optic lobe
ot., otocyst
ot.c., otic cup
ot.p., otic placode
ov., ovary
o.v., omphalomesenteric vein
p., perineum
p.b., polar body
p.b.c., posterior branchial complex
p.c., posterior semicircular canal
p.g., primitive groove
ph.b., pharyngeal bursa
ph.m., pharyngeal membrane
pl., placenta
p.m., parietal mesoderm

pr., pronucleus
p.r., posterior ridge
p.s., peri-intimal space
p.t., pronephric tubule
R., Rathke's pouch
r., rectum
r.b., residual body
s., somite
sac., sacculus
s.c., seam of closure
s.ch., serous chorion
s.e., endolymphatic sac
s.f., spiral filament
s.g., stratum granulosum
s.m., shell membrane
sp., spinal cord
S.p., Seesel's pouch
s.r., sinus rhomboidalis
st., stomach
s.t., sinus terminalis
s.v., sinus venosus
t., tingierbare Körner
th., thymus
thy., thyroid
u., uterus
u.o., urogenital orifice
ur., ureter
u.s., urinogenital sinus
u.s.g., utriculosaccular groove
utric., utriculus
u.v., umbilical vein
v., ventricle
v.a., ventral aorta
v.c.f., vestibulocochlear fossa
v.ch., vascular chorion
v.c., ventral concavity
v.p., ventral pouch
W.d., wolffian duct
x., X-chromosome
y., Y-chromosome
y.b., yolk balls
y.g., yolk granules
y.s., yolk sac
z., zona pellucida

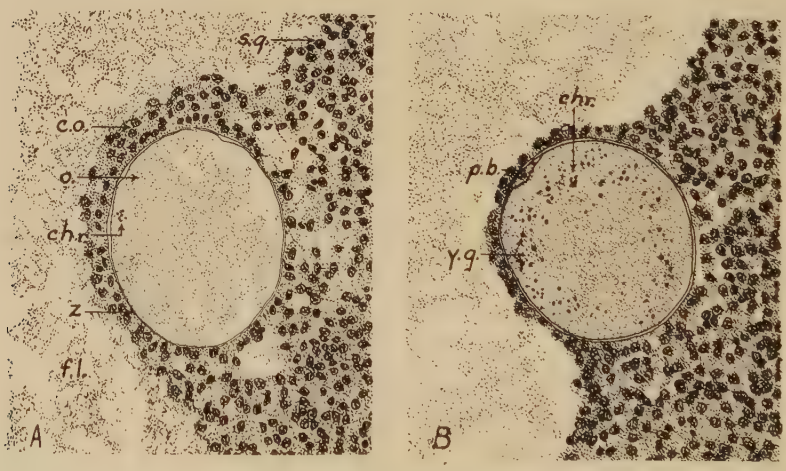


Fig. 1 Ovarian ova. A, specimen 16270[2] showing first maturation division. B, specimen 16274 showing first polar body and the sub-peripheral distribution of yolk granules.

[2] This is the catalogue number of this specimen in The Wistar Institute embryological collection. Throughout this book such numbers are recorded for the convenience of any investigators who may wish to examine the original material.

which is from 0.0006 to 0.004 mm. in thickness, fits closely about the egg cell and the first polar body and presses them tightly together, so that the latter makes an elongated depression on the surface of the egg. Beneath the zona pellucida a radially striated layer some 0.0012 mm. thick is visible in some preparations. Hartman ('16) noticed it, but considered its homology to the zona radiata of other forms uncertain.

The testicular sperm. The spermatogonium (Painter, '22) has twenty-two chromosomes (fig. 2, A), including ten pairs of autosomes, an X-chromosome, and a Y-chromosome. The last two, the sex chromosomes, are visibly represented during

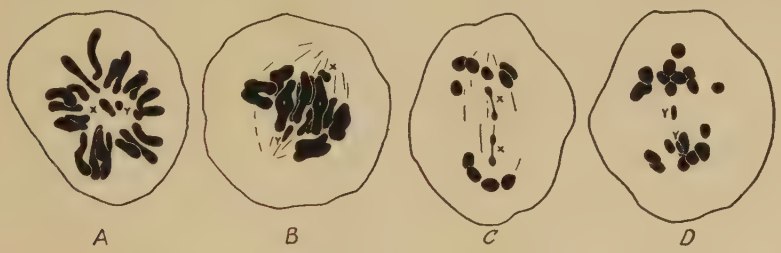


Fig. 2 Spermatogenesis (after Painter, '22). A, polar view of spermatogonium showing ten pairs of autosomes, an X-chromosome, and a Y-chromosome. B, equatorial view of first maturation division showing 12 chromosomes on account of precocious separation of X and Y elements. C, equatorial view of second maturation division showing X-chromosomes. Not all chromosomes are included in this figure. D, equatorial view of second maturation division showing Y-chromosomes. The true haploid number (11) may be counted at the upper pole.

the growth stage of the primary spermatocyte as the chromatin nucleolus (Painter, '24), which is the product of their synapsis. The X and Y components segregate (fig. 2, B) in the first maturation division, so that two kinds of secondary spermatocytes are formed in equal numbers. Both Jordan ('11) and Painter ('22), have recognized a distinct resting stage between the two maturation divisions. In the second maturation division the sex chromosomes divide equationally (fig. 2, C and D).

Spermiogenesis (fig. 3) has been described by Duesberg ('20). Fat droplets are always present in early spermatids.

These later disappear. The tail fiber grows out from two centrioles which are situated near the surface at the posterior of the cell (fig. 3, A). The nucleus migrates to a point opposite the centrioles (therefore at the anterior of the cell) and becomes condensed into a flattened egg-shaped mass in which no details are recognizable. The centrioles, which are connected by a fine fiber, follow the nucleus, and the proximal one becomes attached to it (fig. 3, B). The distal one, which

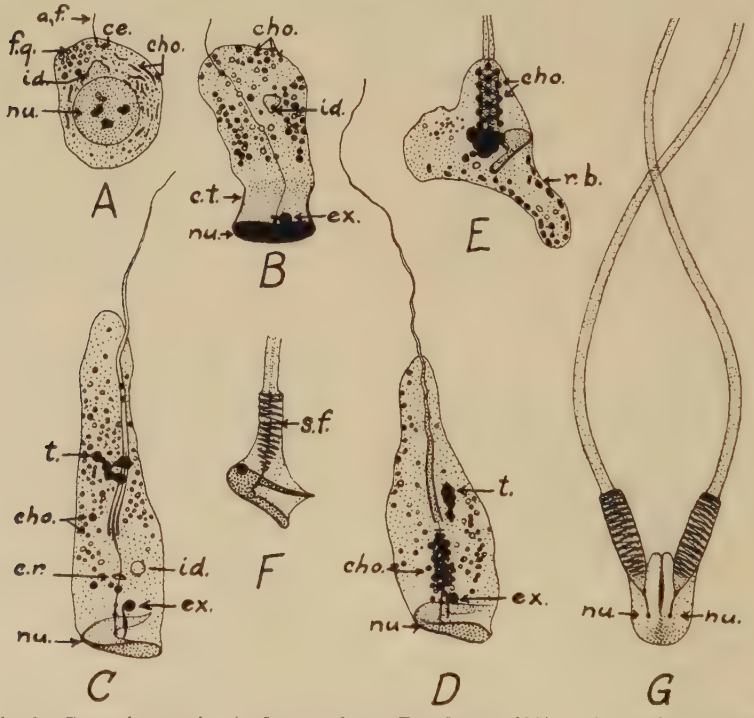


Fig. 3 Spermiogenesis (redrawn from Duesberg, '20). A, early spermatid. The idiozome has already broken and released the two centrioles which are now giving rise to the axial filament. Fat globules are numerous, and are always collected into one group. Chondriosomes are filamentous. B, chondriosomes have become granular. Caudal tube formed. Excrescence still attached to distal centriole. C, the ring has begun its migration. Excrescence attaches separately to swallow-tail nucleus. D, many chondriosomes have collected around axial filament. E, residual body about half formed. F, residual body has been cast off. Chondriosomes have formed spiral around axial filament. Spermatozoon is now free in semeniferous tubule. G, in epididymis the spermatozoa have paired by fusion in the head region along the heavier arm of the V-shaped nucleus. Middle piece now runs between legs of the V instead of at right angles to it.

is still attached to the axial filament, produces an excrescence which becomes quite large and conspicuous. This later degenerates, and its function is unknown. Next, the distal centriole flattens and divides into a proximal granule and a distal ring, the latter encircling the axial filament (fig. 3, C). By this time the nucleus has assumed a curious swallow-tail or V-shape, the proximal centriole being attached at the inside of the angle, and the axial filament extending at right angles to the plane of the V. The ring now moves distally along the axial filament, and as it does so many of the chondriosomes from the surrounding cytoplasm migrate to the axial filament and become attached to that part over which the ring has passed (fig. 3, D). The remaining chondriosomes and the remnants of the Golgi material and idiosome, together with most of the cytoplasm, collect in a residual body anterior to the nucleus (fig. 3, E), and are cast off. At this time the spermatozoon is discharged into the lumen of the seminiferous tubule. Here the chondriosomes which had become attached in the middle piece fuse into a spiral around the axial filament (fig. 3, F); the V-shaped nucleus turns through an angle of 90° so that the middle piece now runs between the legs of the V; and, finally, the spermatozoa fuse together in pairs (fig. 3, G), the line of junction being only in the head region along the heavier leg of the V. These conjugate sperms, as Wilson ('28, p. 305) calls them are a rare phenomenon, but not unique—outside of the marsupials they are found in a beetle, Dytiscus (Ballowitz, 1895), and in a gastropod, Turritella (Retzius, '06). The significance of the pairing is not known. Selenka (1887) pointed out that when the individuals of a pair separate later on they can no longer swim in a straight line, but describe circles. He thinks that most of the spermatozoa remain paired until they have reached the upper part of the oviduct, and then normally separate before fertilization.

Mating.[3] According to Hartman ('23 and '28), the breeding season in Texas begins with the first week of January.

[3] This word is here used only as a synonym for copulation, and does not imply permanent pairing. Any male will copulate with any female in oestrus.

Unmated females show a regular dioestrous cycle of about 28 days, as indicated by urinogenital sinus smears, beginning in January and ending in September. The last 3 months of the year constitute an anoestrum. Ovulation does not occur during lactation. Pregnancy lasts nearly 13 days, and lactation from 70 to 80 days, so that certainly two litters, and possibly three, may be borne by one female in a year. My own experience seems to indicate that the anoestrum may be somewhat longer in Pennsylvania during cold winters, and probably two litters is a maximum for this part of the country.

The graafian follicles usually burst during or very near the time of oestrus. Hartman ('28, p. 162) records one observation which he interprets as evidence that copulation sometimes takes place too late for fertilization (i.e., after the ova have become enclosed in too much albumen, or have begun to degenerate). If so, the implication is that in the opossum ovulation is not dependent upon any stimulus from coitus or related to oestrus in any invariable way. The cleavage stages or early vesicles occasionally found at abnormally long intervals after coitus may be interpreted as confirmatory evidence. Hartman's no. 298 is a case in point. The vesicles he found in the first operation 6 days after coitus belong to my stage 10 and are approximately 2 days late. In the second operation, 9½ days after coitus, he found vesicles of my stage 21. This represents a perfectly normal rate of development between the two operations, so the whole batch of eggs must have started development 2 days late. This means they would have entered the uterus uncleaved about 3 days postcoitum. On March 1, 1937, I obtained eleven uncleaved uterine ova (no. 137) in perfect condition from a uterus opened exactly 73 hours postcoitum. In this case the female had been isolated immediately after the observed copulation, so the possibility of a second copulation 2 days later is ruled out.

Also of interest in this connection is the evidence of a difference in fertility between the matings which occur during daylight hours and those which occur in the night or very early morning. Selenka said that he had never seen mating occur before 7 in the morning or after 11. Though I have seen one mating at 3 o'clock in the afternoon, it is true that it is very exceptional for matings to occur after 11 A.M. On the other hand, the 7 o'clock early limit is quite definitely wrong. By far the majority of all matings occur before that time, and I have some evidence that the matings which occur between 7 and 11 are not really normal.

Of the sixty-one matings which have been observed in my colony after 7 o'clock in the morning, only eleven were fertile. There is thus a ratio of five and one-half infertile matings to one fertile mating after 7 A.M.

Only occasionally have observations been continued through the whole 24 hours, so the total number of matings which occurred before 7 A.M. is not known for any large number of days. But the total number of adult females which were in the colony at any given time is known, and the normal length of the oestrous cycle (Hartman, '23) is known, as already mentioned. If it is assumed for the sake of a calculation that every adult female mated once each cycle except during the annual anoestrous period and the times when she was either pregnant or lactating, the maximum possible number of matings in my colony can be computed for any given length of time. For the year 1935 this number is 209. Since thirty-one matings were observed after 7 o'clock in the morning, there are left only 178 conceivably possible matings which might have occurred before seven. If the total number of litters born during 1935, minus those from matings which occurred after 7 o'clock, be multiplied by $5\frac{1}{2}$, the product in 203.5. This is the number of matings which must have occurred before 7, if the ratio of infertile matings is as high before 7 as it is afterward. But this number is 13% greater than 178, the maximum possible number of matings before 7 o'clock. In other words, it is impossible that the percentage

of fertility was as bad in matings before 7 as it was in those after 7. As the real number of matings was probably much less than the computed maximal possible number, the real percentage of fertility must be very much higher in the earlier matings.

A possible explanation is that ovulation and oestrus both usually occur in the early morning hours (i.e., before 7), and that when mating is delayed it usually means that oestrus is late with reference to ovulation, and correspondingly the sperm reach the ova too late for fertilization. Usually only unfertilized ova are found in females which have been seen to mate after 7.

All the ova from one ovary are discharged simultaneously, and both ovaries ovulate together. The average number of eggs from each ovary at each ovulation is eleven (Hartman, '19), though as many as twenty-two from each ovary have been recorded.

Mature spermatozoa may be found in testes at any time of the year, but maturation stages are more abundant at the beginning of the breeding season (Painter, '22). The male is probably capable of fertile coitus at any time, but is aroused to activity only by the 'heat' of the female.

During copulation the penis (fig. 4) passes directly into the urinogenital sinus (fig. 5). The glans of the penis is bifid, but the urethra opens between, not at the tips of, the prongs. However, a groove, which is very nearly converted into a tube by its overhanging edges, extends along the medial side of each prong for more than half of its length. It is possible that these grooves serve in place of a bifid urethra to carry the semen directly into the lateral vaginal canals.

These latter organs show a considerable muscular activity during oestrus (Hartman, '24). By alternate contraction of their proximal and distal portions they churn the liquid within them. When semen is introduced into this liquid two things happen. In the first place, some spermatozoa are delivered to the os uteri almost immediately by the currents set up by the churning. And in the second place, a coagulation

of the mixture of lateral vaginal canal fluid and semen begins within a very short time. The spermatozoa which do not escape quickly into the uterus are then imprisoned in a coagulated mass—the vaginal plug. Twenty-four hours later the vaginal plug has disappeared.

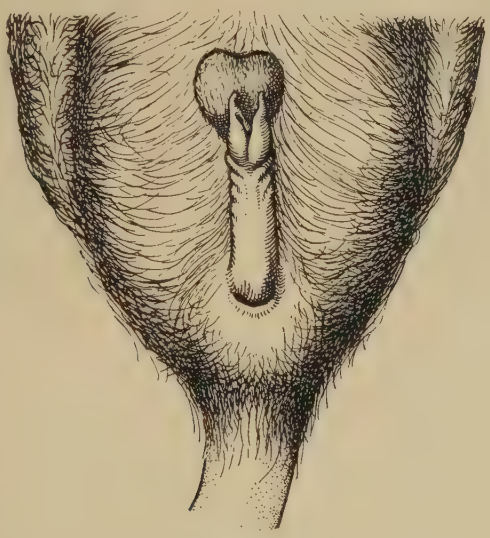


Fig. 4 Ventral view of male external genitalia. The penis is caudal to the scrotum, and its glans is bifurcate. During detumescence it retreats out of sight to a position similar to that of the clitoris.

Lewis ('25) studied the bacteria present in these canals at different stages of the oestrous cycle. During the anoestrum he found their number to be reduced to a minimum. During the prooestrum the numbers, both of individuals and of species, increase rapidly. The maximum is attained at oestrus. As all of the species identified, of which there are about a dozen, are well-known saprophytes, he concluded that they are responsible for the removal of the vaginal plug and for the general cleaning of the canals during the dioestrum and anoestrum.

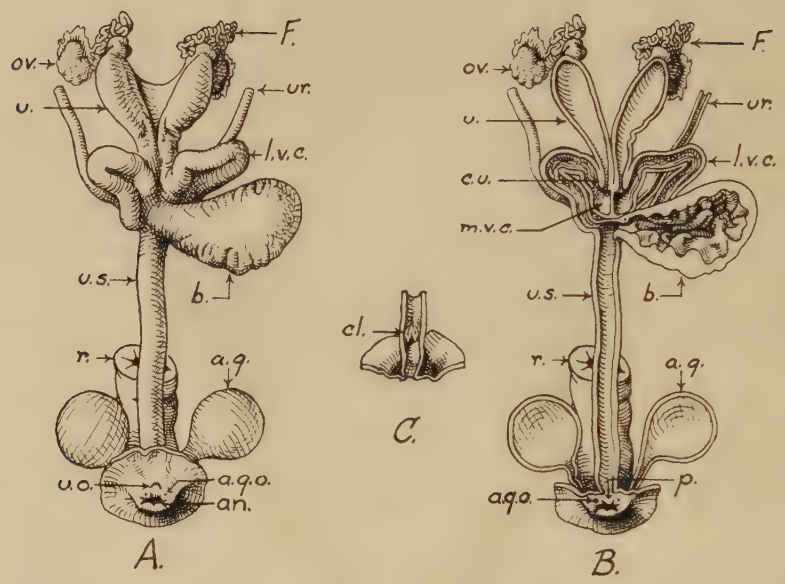


Fig. 5 Female urinogenital system. A, ventral view of adult organs in anoestrous condition. B, dissection of the same specimen. The uteri are seen to be completely double, each protruding into its own median vaginal cul-de-sac. From this median position the vagina runs anteriorly and laterally, and then turns back upon itself to empty into the urinogenital sinus immediately dorsal to the neck of the bladder. The ureter on each side passes between the arms of the lateral vaginal loop to enter the bladder through a prominent papilla on the dorsal side of the neck of this organ. The urinogenital sinus and the rectum open to the exterior separately, but very near each other. There is no cloaca. The anal musk glands, which are here shown fully distended, open exteriorly through two very small pores. C, the ventral wall of the urinogenital sinus shows a bifurcate glans clitoridis. The skin fold beyond this point is the prepuce.

II. THE FIRST DAY

Stage 1. Fertilization. The tubal ovum.

Fertilization. As the ovum can descend the oviduct in as short a time as 24 hours (Hartman, '28), and as it comes to be surrounded by an impenetrable layer of albumen and a thin shell membrane while doing so, and as unfertilized eggs begin to degenerate within 24 hours (S. C. Smith, '25), it is necessary for the spermatozoon to find the ovum at the extreme upper end of the oviduct, and soon after ovulation, if fertilization is to be accomplished. But ovulation does not bear a precise or consistent time relation to copulation. Some-

times, apparently, the ova are discharged more than 12 hours before copulation, and on these occasions the mating is infertile.

The sperm twins always separate before fertilization. This is indicated by the fact that the sperm found entrapped in the albumen of the egg are always single. The actual entrance of the spermatozoon into the ovum has not been observed in any marsupial.

In summary: Occasionally precocious ovulation results in sterile mating. Occasionally, perhaps, delayed ovulation results in a post-oestrous period. Most often ovulation occurs within a few hours before or after copulation, and fertilization by single spermatozoa takes place in the upper part of the fallopian tubes within a few hours after ovulation.

Stage 1

The tubal ovum. From about the twelfth hour to the thirty-sixth hour after coitus one may expect normally to find the ova in the tubes. It is possible that immediately after dehiscence of the follicle the granulosa cells are adherent to the zona pellucida for a short time, but, if so, they are very soon shed and the ovum is left covered only by the zona. In some way (perhaps by a change in osmotic pressure in the surrounding fluid) the volume of the egg is slightly reduced at this time (Hartman, '19). Its new dimensions are 0.122 mm. for the longest diameter, and 0.104 mm. for the shortest (fig. 6).

After the formation of the first polar body in the ovary the chromosomes apparently do not enter a resting stage as they do at the corresponding stage of spermatogenesis (vide, p. 17), but immediately arrange themselves on the spindle for the second maturation division. This is the condition in the early tubal ovum (fig. 6). If fertilization does not take place, the second maturation division does not complete itself, though the X-diad, which is sometimes precocious, may divide. The other chromosomes remain in a metaphase distribution during the entire descent of the tubes (24 hours).

At the end of this time all chromosomes show signs of degeneration, and the egg undergoes irregular fragmentation without mitosis.

If fertilization has occurred, the second maturation division giving rise to the second polar body takes place. In the normal metaphase of this division there are at first eleven diads; but the X-diad often divides precociously, so that spindles are frequently found showing ten diads and two monads. This led Hartman ('19, p. 35) to the conclusion that the haploid number of chromosomes is twelve. Painter

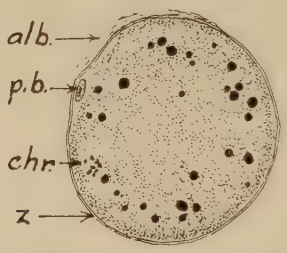


Fig. 6 Early tubal ovum (16266). Albumen is just beginning to be deposited on the zona. There is still only one polar body. The organization of the cytoplasm into a shallow, peripheral, homogeneous, darkly-staining zone; a subperipheral, irregular, yolk-laden zone; and a broad, homogeneous, lightly-staining, central zone; is well shown. The yolk granules are larger than they were in the ovarian ovum.

('22, p. 35) after a study of spermatogenesis, where a parallel precocious splitting of the sex chromosome often occurs (v, fig. 2, B), noted this mistake and corrected it.[4]

The two polar bodies are visible in many of the very early cleavage specimens. Hartman ('19) has seen what he interprets as polar bodies as late as the 34-celled stage; but usually one finds them difficult to recognize after the second cleavage on account of the phenomenon of yolk extrusion, which will be described below.

[4] To anyone interested in the source papers one further explanation is necessary. At the same time that Painter corrected Hartman's original mistake he inadvertently introduced another which makes his account somewhat puzzling. He consistently refers to the diads in Hartman's figures of second maturation divisions as tetrads, and to the monads as diads. I have pointed this out to him in conversation, and he agrees that it was a slip of the tongue.

Soon after the ovum enters the tube albumen begins to be deposited upon the zona in thin concentric sheets, which sometimes entrap numerous spermatozoa and occasionally a few epithelial cells from the oviduct. The stratification of the albumen seems to be due to the rolling of the egg during its descent of the oviduct. The final thickness of the albumen before resorption begins is some 0.25 mm., though there is considerable variability in this dimension.

Just before the egg passes into the uterus a shell membrane is secreted upon the surface of the albumen by the shell glands of the lower part of the oviduct. These glands have been seen by Hartman ('16) and Anderson ('28). The shell is about 0.001 mm. in thickness when the ovum reaches the uterus (Hartman, '16). The diameter of the egg including ovum, albumen, and shell is about 0.6 mm. The shell is leathery rather than brittle, and seems, as evidenced by staining reactions, to be composed of two quite different materials. If not overstained, a clear, yellowish, homogeneous, non-chromophilic matrix may be distinguished as the principle substance of the shell. Within this, and usually confined to its inner half, is a granular, intensely basophilic, less flexible deposit. When the albumen beneath the shell shrinks during dehydration the shell wrinkles, and the darker material frequently cracks up into many small pieces which remain embedded in the more flexible yellowish material. I mention this detail because I have no doubt that patches of this dark-staining, granular material were what Selenka (1887, plate XVII) mistook for remnants of the nuclei of granulosa cells.

Soon after the formation of the second polar body both pronuclei may be seen in the resting stage near the surface of the egg and surrounded by a relatively yolk-free region of cytoplasm. The yolk in other parts of the egg retains its typical sub-peripheral distribution, but the granules and droplets have fused into fewer elements which are of correspondingly larger size. The two pronuclei now migrate toward the center of the ovum carrying their yolk-free area of cytoplasm with them (fig. 7). As they pass within the limits of the

spherical yolk layer, this latter comes together again behind them. In this condition the ovum reaches the uterus.

III. THE SECOND DAY

Stage 2. The first cleavage. The extrusion of yolk.

The first cleavage. During the first half of the second day after mating the fertilized ovum reaches the uterus in the

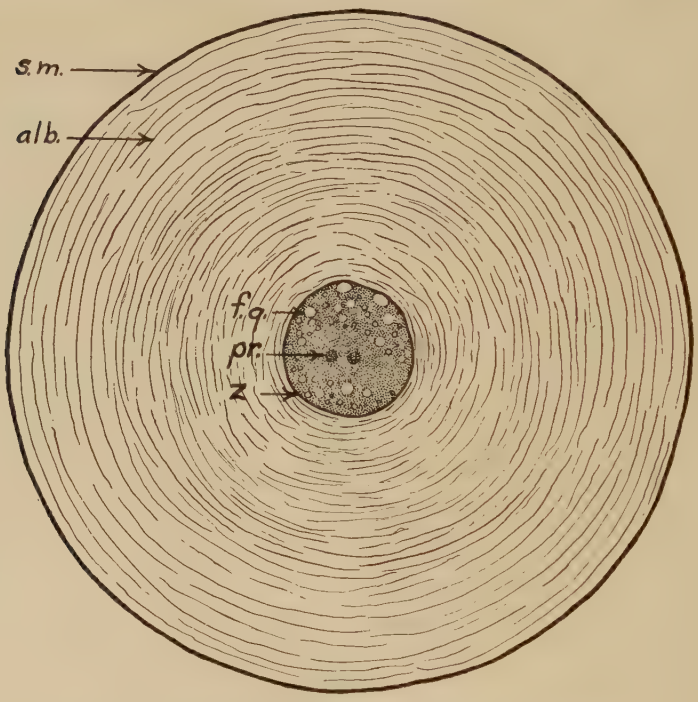


Fig. 7 Ovum with complete albumen layer and shell membrane (× 150). The pronuclei have migrated to the center of the ovum. In this figure the ovum proper is taken from specimen 16244; but the dimensions of the albumen and shell represent the average dimensions in fresh specimens. After dehydration the albumen shrinks considerably and the shell wrinkles (see fig. 10).

pronuclear stage. The fusion of the pronuclei and the formation of the spindle for the first cleavage occur at the center of the egg. I have studied two eggs which show this cleavage in process. The first is a late anaphase (no. 16265)

(fig. 8). The ovum has become very elongate and equatorially constricted. The chromosomes are grouped about two centrosomes at opposite poles, with the exception in each group of one chromosome which lags nearer the equator. This lagging chromosome is much nearer the equator in one case than in the other. Indeed, a closer inspection reveals that all the chromosomes on one side of the equator are at a slightly different stage of development from those on the other, those approaching one pole (the lower pole in fig. 8, B) being nearer the telophase condition than those approaching the other.

Fig. 8 Late anaphase of first cleavage from one of Hartman's specimens (16265). A, the entire ovum showing elongation and equatorial constriction of the cytosome, much deutoplasmic material in the cytoplasm, the beginning of yolk extrusion in the lower hemisphere, and the situation of the spindle a little above center. B, enlarged view of the spindle to show the difference in stage of vacuolation of the chromatin at the two poles.

Furthermore, the yolk is not equally divided between the two poles. A count of the fat droplets in camera lucida tracings of serial sections of this specimen reveals that about 60% of them are in that half of the egg which has the more advanced chromosomes. This is not a difference in concentration of yolk, but merely in volume of cytoplasm plus its inclusions at the two poles. The location of the spindle and the plane of division are off center so that the two daughter cells will be unequal in size. These early indications of a difference between the two poles of the egg are of interest as they foreshadow the fact that the cells at one pole of the prospective blastocyst will divide more rapidly than those at

the other, and that these cells are descended from the larger of the first two blastomeres.

The second specimen showing the first cleavage in process is a telophase (no. 16256). The equatorial constriction of the cytosome is not yet complete, but the wells of the nuclei have formed. The only indication of polarity recognizable at this stage is a difference in size of the two prospective daughter cells. With reference to the polarity indicated in these two specimens the plane of the first cleavage is equatorial.

The extrusion of yolk. A very extraordinary change has taken place in the cytoplasm while the nuclear events just described were in progress. It was observed in the last chapter that during the passage of the ovum down the tubes the yolk granules and fat droplets collect into fewer units of larger size. The next change which can be recognized takes place when the pronuclei are approaching each other in the center of the egg and before the dissolution of their membranes. At this time a few large fat droplets can be seen protruding from the surface of the egg, as if they were being slowly extruded. That they meet considerable resistance at this surface is witnessed by the degree of distortion they undergo. The globules below the surface are spherical, but those at the surface are invariably much flattened—more, probably, than the laws of capillarity would account for. The resistance responsible for the compression is in part due to a true cell membrane, but the zona pellucida is still in contact with the surface of the ovum and may participate in this resistance. The globules apparently burst through the surface of the ovum, and their contents are dissipated into a space which now arises for the first time between the zona and the egg proper. This perivitelline space first appears only in the equatorial region when the ovum elongates during the first cleavage.

But not only fat globules are extruded. A little later, as the first cleavage furrow sinks into the ovum, large balls of cytoplasm including numerous granules of yolk from the subperipheral zone are forced through the surface and deposited

in the perivitelline space (figs. 8 and 9). These balls contain no chromatin, of course. They vary considerably in size, the largest being sometimes 0.030 mm. in diameter. The extrusion of fragments of this size makes a drastic reduction in the volume of the ovum proper. Such extrusion seems to begin at the more advanced pole (the lower in all my figures), but I have not been able to discover any considerable difference in the eventual extent of this process at the two poles.

The probable explanation of the difference in size of the first two blastomeres is that the nucleus of one is formed earlier than the nucleus of the other. This means that the process of gelation associated with this mitotic pole is in advance of that associated with the other, and accordingly,

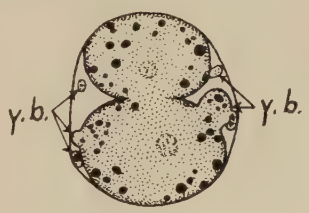


Fig. 9 Late telophase of first cleavage (16256) showing yolk extrusion.

comes to involve or appropriate a larger portion of the available cytoplasm. This difference in stage of the two poles is recognizable in specimen 16265, as has been pointed out in a preceding paragraph.

The first cleavage is completed about 30 hours after the ovum has entered the uterus in the pronuclear stage, and therefore about 54 hours post coitum.

IV. THE THIRD DAY

Stages 3 to 8. The second cleavage. The third cleavage. The fourth cleavage.

Stages 3 and 4

The second cleavage. The inequality in size of the first two blastomeres is about the same as the inequality in yolk distribution noted at the poles during the first cleavage. The

smaller blastomere averages about 0.082 mm. × 0.061 mm. in its longest and shortest diameters, respectively; whereas the larger averages 0.099 mm. × 0.070 mm. These measurements indicate a discrepancy of about 26% by volume, the larger blastomere containing 63%, the smaller 37% of the total volume of the egg. This information is useful in solving the question of cell lineage at the next stage.

The nuclear difference between the two poles of the egg reappears when the spindles form for the second division. The two eggs of The Wistar Institute collection which show these spindles (no. 16258 and no. 16275) are both anaphases, and in

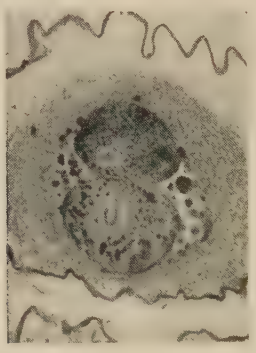


Fig. 10 Section of 2-celled ovum 16258. Photograph from Hartman ('19). Showing spindles for second cleavage, and yolk granules in the perivitelline space.

each case the chromosomes in the larger blastomere are further advanced than those in the smaller. Furthermore, the spindles are not parallel in the two blastomeres, but are approximately at right angles to each other. Both are also at right angles to the position of the original spindle in the first cleavage (fig. 10).

From this point on for the sake of brevity I shall call the larger blastomere of the 2-celled stage A and the smaller B. Before the cleavage furrow has appeared in B, A divides meridionally, so that a 3-celled stage (stage 3) is formed, composed of A_1, A_2 and B. B then divides producing a 4-celled stage (stage 4) which, in accord with the difference

in spindle orientation mentioned above, is crossed like that of the Eutheria.[5]

The evidence for this account of the second cleavage is derived from a study of the volumes of the blastomeres in the 2-, 3-, and 4-celled stages, and from glass plate and wax plate reconstructions of the spindles in two 2-celled eggs. In all, six 2-celled eggs, two 3-celled eggs, and seventeen 4-celled eggs were studied.

It was mentioned above that in the 2-celled stage blastomere A contains about 63% of the total volume of the egg, and blastomere B about 37%, or roughly, 60% and 40%, respectively. In the 3-celled egg (stage 3) there is one large blastomere representing about 50%, and two smaller blastomeres representing about 28% and 22%, respectively, of the total egg volume. The actual volume of this large blastomere is about 229,859 μ^3, which is the same as the volume of blastomere B of the 2-celled stage; but this volume now represents 50% instead of 40% of the total egg volume. The explanation of this is that the total egg volume has been reduced by 10% through yolk elimination, and that this reduction has taken place almost exclusively in the dividing blastomere. The identification of this large blastomere of the 3-celled stage would be reversed if it were true that yolk elimination takes place in resting cells and not in dividing cells; but the evidence from the slides uniformly indicates that the opposite is true. Deutoplasmolysis, as Hill ('10) has named this process, makes its initial appearance when the pronuclei approach the center of the egg and the formation of the mitotic spindle begins. It reaches its climax during cytokinesis. It subsides during the resting stage. It seems reasonably certain, therefore, that the large blastomere of the 3-celled stage which has the same

[5] In the fairly closely related Australian marsupial Dasyurus viverrinus Hill ('10) found that this celavage always results in a radial 4-celled stage instead of a crossed one. Selenka (1887) figured the 4-celled stage in the opossum as radial, but more recent studies on a large number of specimens by Hartman ('16, '19) and Smith ('25) show conclusively that in the opossum such radial 4-celled stages are always abnormal and are usually not cleavage stages at all, but fragmented, unfertilized eggs.

volume as blastomere B of the 2-celled stage is actually blastomere B still undivided, and that the two smaller cells are the descendants of blastomere A, which lost 51,079 μ^3 of its volume by discharging that amount of yolk into the 'perivitelline' space during division.

The 4-celled stage (stage 4) is produced by the division of blastomere B. It divides meridionally, but at right angles to the plane of division of A; and, in doing so, it in turn discharges yolk while blastomeres A_1 and A_2 remain quiescent. In this manner there is a restoration of the original volume relations of the A and B poles of the egg. That is to say, in

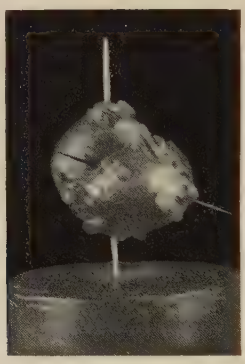


Fig. 11 Photograph of wax model of 16275, showing yolk granules protruding through the surface of the blastomeres. The needles run through the poles of the mitotic spindles and show their angle of inclination to each other.

the 4-celled stage the daughter cells of A taken together again represent about 60% of the total volume of the egg; and those of B about 40%.

It should be mentioned that Hartman ('19) believed that the spindles in A and B are parallel when they first form, and subsequently rotate during division. His opinion was based upon an egg (no. 306.2 of the Hartman series, now no. 16275 of The Wistar Institute collection) in which he considered the spindles to be parallel. I have made both a glass plate reconstruction and a wax plate reconstruction (fig. 11) of this egg, and both show this not to be the case. The

spindles are in reality inclined at an angle of about 70°. But one spindle is in an early anaphase (that in B), whereas, the other shows all the chromosomes already at the poles (that in A), and the plane of section is such as to make interpretation without reconstruction a little difficult. All of the spindle in B and one pole of that in A may be seen in the same three sections. The other pole of the more advanced spindle in the larger blastomere is several sections below, and might conceivably have been overlooked. At any rate, in reconstruction it is very clear that the spindles in this egg are much nearer to being at right angles to one another than they are to being parallel.

My own interpretation of this spindle orientation is that it is the mechanical result of some fairly simple pressure relations which need not involve any rotation of spindles or blastomeres. Since the spindle in A forms first, the process of gelation which is associated with the poles of the mitotic spindle is more advanced in this blastomere than in B. The denser spheres of protoplasm about the poles of A during its elongation exert a pressure upon the more liquid blastomere B just at the time when the spindle is beginning to form in the latter. As spheres of gelation arise and enlarge in B they can find room only in the direction where the least pressure is applied by the already elongated blastomere A. This means in a direction at right angles to the long axis of A, for both blastomeres are surrounded and mutually appressed by the zona pellucida.

Like the first cleavage, the second is slightly unequal, so that A_1 is slightly larger than A_2, and B_1 is slightly larger than B_2.

In summary: The larger of the first two blastomeres, A, divides first in a meridional plane, producing two slightly unequal daughter cells, A_1 and A_2, and losing about 10% of the total egg volume by yolk elimination. B then divides, also in a meridional plane but at right angles to the plane of division of A, also unequally, and also losing volume by yolk elimination.

Stages 5 and 6

The third cleavage. At the end of the 4-celled stage (stage 4′) the total mass of the blastomeres has been reduced by the elimination of deutoplasm to such an extent that they no longer fill the space within the zona pellucida, but are separated from one another and to some extent from the zona by yolk granules (fig. 12, B). After this there is little further yolk extrusion.

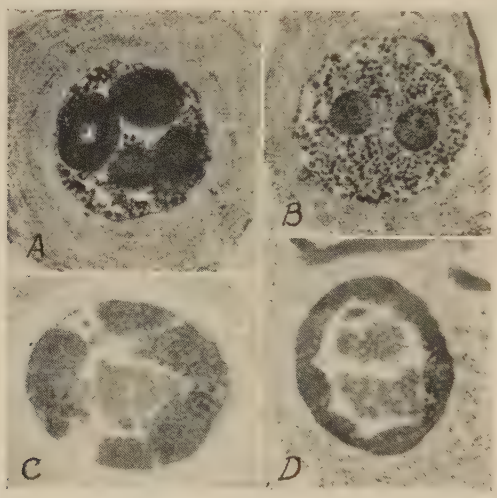


Fig. 12 Photographs from Hartman ('19). A, early 4-celled stage showing large blastomeres in contact with zona. B, late 4-celled stage showing blastomeres much reduced by yolk extrusion. C, 14-celled protoblastocyst with yolk showing liquefaction. D, 32-celled complete unilaminar blastocyst.

The larger cells at the A-pole of the egg continue to divide precociously. A 5-celled stage is formed by the division of A_1. This is followed quickly by a 6-celled stage after division of A_2. In stage 5 the two larger cells are B_1 and B_2 still undivided. The larger cells, therefore, are now at the B-pole instead of the A-pole of the egg. In the 8-celled stage (stage 6) the A-pole again contains the larger cells (on account of the division of B_1 and B_2), though the difference is less dicided than it was originally.

Stages 7 and 8

The fourth cleavage. Eggs containing very nearly every number of blastomeres from one to thirty-two (a fully formed unilaminar blastocyst) were collected by Hartman and left here at The Wistar Institute. This collection has been the basis of a detailed study of cell volumes which I have made in an effort to discover the fate of the first two blastomeres. The identification of each individual cell can be followed as far as the 12-celled stage with accuracy.

At the end of the third cleavage (stage 6, no. 16232) there are four large cells and four smaller cells. The four large ones are at the A-pole. In the 10-celled ovum (16233a) there are six large cells and four small ones. This is because two of the large cells at the A-pole have divided producing the four smallest cells, and the remaining two at the A-pole plus the four at the B-pole are now classified as large by contrast. In the 12-celled ovum (stage 7, no. 16233b) there are four large and eight small cells. This is because the two remaining A-cells have divided, making eight small cells at the A-pole, and the four B-cells are still undivided. Hartman ('16, p. 32) figured wax models of the 10-celled and 12-celled ova here referred to.

Mitotic figures in the 12-celled specimen indicate which cells are prepared to divide next. The cells showing these figures are two of the large B-cells. I do not have a 14-celled egg, but according to these mitotic figures the 14-celled stage must be composed of two cells at the B-pole which belong to the fourth generation, and twelve smaller which belong to the fifth generation. The 16-celled stage (stage 8, no. 16229) seems to be produced, not by the division of the remaining two large cells, but by the fifth cleavage beginning at the opposite pole before the fourth cleavage has completed itself at the B-pole. This is inferred from the fact that two small cells are found at the A-pole, the two large cells can still be seen at the B-pole, and twelve intermediate cells are arranged between, of which twelve probably four belong to the B-group and

eight to the A-group. Since each of these cell divisions is slightly unequal, there is a fairly evenly graded series of cell sizes from large to small. I do not mean, of course, that they are lined up according to graded sizes; actually, the two B-cells which we saw in mitosis at the 12-celled stage form four small cells at the B-pole near the remaining two undivided and therefore large B-cells. The two smallest cells are at the opposite pole. The intermediate ones cannot be dependably distinguished into A and B groups. Furthermore, the last large cells at the B-pole soon divide, making it impossible immediately thereafter to be sure where the B-pole is.

There are three factors which together make the further tracing of cell lineage impossible: 1) the daughter cells from each division are slightly unequal; 2) all the cells become so small that the inherent margin of error in the wax plate method, introduced by the square corners of the individual wax sections, becomes proportionately too great for the method to be practical; 3) the cells begin to resorb yolk and to grow, so that daughter cells attain the size of their parent cells before they divide again. Hartman also found it impossible to recognize any polarity or to identify the individual blastomeres immediately after the 16-celled stage.

However, before two more cleavage waves have passed (i.e., in the 50- to 60-celled ova) a new polarity appears, which is permanent. It is naturally of interest to inquire whether the original polarity of the ovum is related to the secondary polarity of the blastocyst, and, if so, how. The evidence bearing upon this point will be discussed in chapter V.

V. THE FOURTH DAY

Stages 9 to 12. The unilaminar blastocyst. The endoderm mother-cells. The new polarity. The primitive endoderm. The medullary plate. The beginning of expansion of the vesicle.

Stage 9

The unilaminar blastocyst. No true morula is formed. It was pointed out in the last chapter that at the 4-celled stage

the blastomeres lose contact with each other as a consequence of the elimination of a considerable quantity of yolk (stage 4′). This extruded yolk lies between them and around them, and within the zona pellucida practically all the space not occupied by the blastomeres is filled with yolk granules (fig. 12, A). The blastomeres themselves have hardly become separated from each other before some of them begin to flatten out against the zona. Those which are not in contact with this membrane are spherical. Those which touch it flatten out against it. Even in the early cleavage stages (stages 5 and 6 and fig.12, B) this leads to some semblance of blastocyst formation. By the 16-celled stage (stage 8) nearly all of the blastomeres are somewhat flattened against the zona, though the wall of the blastocyst is not yet complete and a few spherical cells which are not yet in contact with the zona may still be found. By the time there are thirty-two cells (stage 9 and fig. 12, D) all the gaps in the wall are usually filled, and the unilaminar blastocyst is complete.

This flattening out of individual cells against the zona pellucida before there is any such thing as a blastocyst wall, and when no morula stage has been formed, seems to be clear evidence that the accumulation of fluid in spaces between the cells of the morula by endosmosis is not the causative factor in blastocyst formation. It seems necessary to think of stereotropism as a more likely factor. And in this light, perhaps an important function of the zona is that of providing the proper stimulus for the tropism which leads to blastocyst formation. The yolk granules, of course, not exhibiting any such tropism, are gradually crowded away from the zona by the cells and thus passively come to occupy the cavity of the blastocyst.

So far the total volume of the egg proper has not increased. The zona pellucida has become thinner until it serves only to give a progressively diminishing distinctness to the inner border of the albumen. The albumen layer, if it has changed at all, is slightly thicker. The shell membrane, however, has definitely increased in thickness, measuring now between

0.003 mm. and 0.004 mm. All of the eggs roll or float about freely in the uterine fluid. Hartman's litter no. 314 (e.g., W.I.C. 16220), removed $3\frac{1}{2}$ days after copulation, are type specimens for stage 9.

Stage 10

The endoderm mother-cells. During the second half of the fourth day when the total number of cells in the blastocyst is between fifty and sixty some extraordinary events occur which were first noticed by Selenka (1887) and then more completely worked out by Hartman ('19).[6] Certain cells in

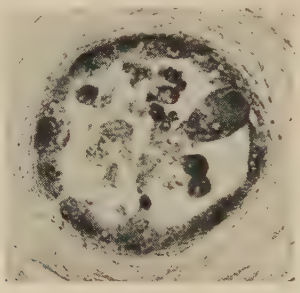


Fig. 13 Photograph of section through 16284, showing endoderm mother cell. From Hartman ('19).

one-half of the blastocyst which may be called the endoderm mother-cells (Urentodermzellen of Selenka) begin to enlarge enormously (stage 10 and fig. 13). When I first examined them I wondered whether they were just cells of an earlier generation which had remained large while their neighbors became reduced in size by continued division. But a study of the volumes of the cells of the cleavage specimens showed that these endoderm mother-cells are larger than any of the

[6] Hill ('10) denied the morphological significance which Selenka had attributed to the cells about to be described here, but at that time Hill had not seen any Didelphys preparations, and his opinions were based only upon his data from Dasyurus. Hartman ('16) following Hill's lead, made the same denial, but later ('19) he confirmed Senlenka's interpretation and gave a very complete and perfect account of the origin of the endoderm.

cells in the immediately preceding stages, so that they necessarily have attained this size by growth, not by remaining undivided. For instance, in specimen 16218 there is a single endoderm mother-cell the longest diameter of which is 60 μ, the shortest 42 μ. There has not been a cell of this size in the egg since the two B-cells of the 6-celled stage divided. The average endoderm mother-cell in the early stages of endoderm formation is about 46 μ × 35 μ in its longest and shortest diameters respectively. This is about the size of the four large A-cells in the 8-celled stage. The last of these four is present in the 11-celled egg. From the 12-celled stage until the first endoderm mother-cell appears (50- to 60-celled stage) the cells become progressively smaller. Accordingly, it is clear that the large size of the endoderm mother-cells is due to a rather sudden and exceptional growth on their part.

Being hemmed in laterally by the surrounding cells and peripherally by the zona pellucida, which is still distinctly visible though thin, the endoderm mother-cell as it enlarges extends itself principally in a median direction. In other words, it juts conspicuously into the blastocoel. When it attains its maximal size it usually rolls out of the protoderm (the primitive pluripotential layer from which all the germ layers are derived) and into the blastocoel. Sometimes, however, it divides first; and occasionally two divisions occur before the base of the original cell loses contact with the cells remaining at the surface. This produces for a time a chain of cells projecting into the blastocoel. But in any case, the cells soon free themselves from the protoderm altogether. When free in the blastocoel these endoderm mother-cells are perfectly spherical. They show no consistent peculiarities except those of size and the habit of leaving the blastocyst wall. Some, but not all, stain a little darker than the other cells.

The gap left at the surface by the inward migration of this large cell is soon effaced. Selenka considered such a gap the homologue of the blastopore; and though he recognized that the gap itself is very transitory (1887, p. 114) he described

and figured a slight depression which, he says, for a long time marks the site of the blastopore. In a study of about 100 vesicles of the appropriate stages neither Hartman nor I have been able to find this.

The new polarity. As all of these cells arise in one-half of the blastocyst, a polarization of the vesicle is again recognizable. Accordingly, though it will involve a slight anticipation of details to be described more fully in the later sections of this chapter, this seems to be the logical point at which to discuss the possible relation of the polarity of the cleavage stages to the polarity of the late blastocysts.

Hartman's opinion on this question was that the larger of the first two blastomeres is probably the one which divides more slowly, giving rise to a pole of large and primitive cells which eventually become the 'embryonic area.' The endoderm mother-cells, he supposed, arose from this half of the vesicle before the so-called 'embryonic area' was sharply defined. Shortly thereafter, this area lagged so many generations behind the more rapidly dividing 'trophoblast' that it came to be sharply demarcated from the latter.

There are several difficulties with this interpretation. The first is that it does not account for and is not compatible with the loss of all visible polarity in the early unilaminar blastocyst, which Hartman described. If the larger of the first two blastomeres and its descendants are retarded in cleavage rate, they will always remain larger than the rapidly dividing descendants of the other blastomere which was smaller even to begin with.

This first objection, however, is obviously met by my own studies of cell volume which showed that (as in the pig, vide Heuser and Streeter, '29) the larger of the first two blastomeres is actually the first to divide, and that it, not the smaller one, gives rise to the pole of rapidly dividing cells. This very clearly accounts for the temporary loss of size distinctions among the cells in stage 9; for if the more rapidly dividing cells are larger to begin with, they must necessarily pass through a stage in which they are indistinguishable in

size before they eventually become noticeably smaller than the retarded cells at the opposite pole.

However, even with this correction the attempt to relate the early polarity to the later polarity meets further difficulties. If, for instance, the difference in rate of cell division at the two poles of the cleavage stages be considered to persist long enough to be responsible for the thickness of the 'embryonic area' in contrast to the thinness of the 'trophoblastic area,' this means that the cells of the 'embryonic area,' which have divided more slowly according to the theory, and belong to an earlier cell generation, must correspondingly be fewer in number. Hartman's own cell counts show this not to be the case.

Hartman's counts in four blastocysts of what I call stage 11 show an average of 93.5 cells in the 'embryonic area' and ninety-six cells in the 'trophoblastic area' ('19, p. 62), which is practically identical. The average number of endodermal cells in these same vesicles is thirty-three. If these come from the 'embryonic area' as he believes, then instead of fewer cells in this area there are at least 30% more than in the 'trophoblast.' Or if his theory be modified so as to have them come from the 'trophoblastic area,' their excess would not be enough to indicate a difference of even 1-cell generation. The only possible way to reconcile these figures with the theory of a persistent difference in rate of cell division at the two poles is to assume that the trophoblastic pole is constantly contributing cells to the embryonic pole (just the opposite of what Heuser and Streeter, '29, assume to be the case in the pig).

One other point which must be mentioned is that though so much depends upon this assumption, the cells of the 'embryonic area' have never really been shown to be larger than the cells of the 'trophoblast.' In cross sectional view they are definitely thicker (at least in some stages), which means that their lateromedial dimension is greater. On the other hand, in surface views of whole mounts they appear definitely smaller, as their two surface dimensions are less. This has been shown in the figures by Selenka, Hartman, and myself.

They are, then, larger, in one dimension and definitely smaller in two others, and their actual volumes have not been studied.

Personally, I feel that the source from which all this theorization springs—namely, the idea that the first two blastomeres represent in any significant way the 'embryonic' and 'non-embryonic' portions of the later vesicles—is unsound. In the next few pages I shall suggest a totally different interpretation of the polarization of the later blastocysts.

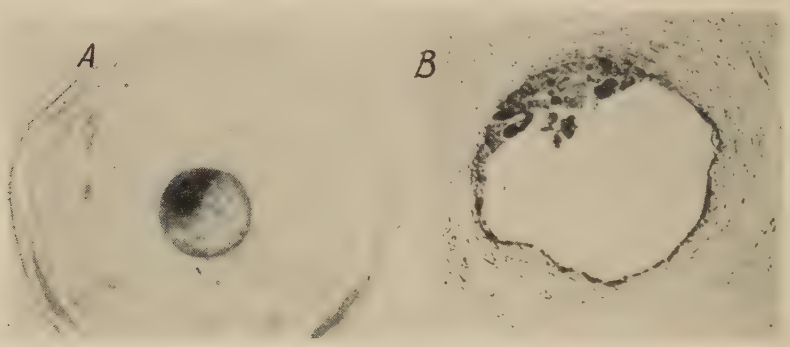


Fig. 14 Photographs of stage 11 (Hartman, '19). A, living ovum in Ringer's solution. B, section through primitive endodermal cells and medullary plate.

Stages 11 and 12

The primitive endoderm. In the 1-celled stage the ovum measured about 0.11 mm. in diameter. No enlargement at all occurs until the endoderm mother-cells begin to form. The principal changes up to this time have consisted of the extrusion of most of the deutoplasm, the segmentation of the protoplasm proper, the orientation of the segments or cells along the zona leaving the yolk balls in the center, the liquefaction and resorption of most of the yolk. These are changes in the distribution and condition of materials contained in the original unicellular ovum.

When the endoderm begins to differentiate, changes which involve the acquisition and utilization of new materials are initiated. Such changes are reflected in the growth of the

blastocyst and the reduction of the albumen. Both of these processes are more conspicuous in the events of the fifth day, but their first stages can be seen in vesicles approximately 4 days old.

Among these vesicles, which are from 0.20 to 0.34 mm. in diameter, the endoderm mother-cells have undergone further differentiation. The spherical endoderm mother-cells, which were free in the blastocoel, soon come into contact with the external layer of protoderm. They then flatten out upon it just as the spherical blastomeres in early cleavage stages flatten out upon the zona pellucida, and doubtless as a result of the same reaction—stereotropism. When more or less flat and in contact with one another or with the protoderm they are called primitive endoderm cells (stages 11 and 12 and fig. 14). These cells multiply mitotically and soon accumlate to a thickness of two or three cells immediately under the protoderm, which at the same time thickens throughout the region that they touch.

The medullary plate. The most interesting and unexpected fact that I have come across in my study of the embryology of the opossum is that the dense plate of cuboidal superficial cells thus formed is a medullary plate (McCrady, '37). In the light of the embryology of all the lower vertebrates this is exactly what one should expect to find; but in the opossum this structure was observed 50 years ago by Selenka (1887) and has since then been seen in many other marsupials, and has been studied by many investigators, all of whom regarded it as an 'embryonic area.' In other words, it has always been supposed that all the embryonic ectoderm (epidermis as well as nervous system) is derived from this plate, and that the ectoderm outside this region gives rise to nothing but extra-embryonic membranes. As a matter of fact, none of the epidermis is derived from this plate, and the extra-embryonic ectoderm is not in any way demarcated from that which will produce body epidermis. The previous interpretation was based upon analogy with the supposed separation of embryonic and non-embryonic or trophoblastic cells in the

eutherian vesicle, and was possible, I believe, only in the absence of a complete series of sectioned specimens. In a study of external views it is easy to be deceived on this point, and, for my own part, I accepted the old interpretation without misgiving until I had studied sections of stage 22 (specimen 16139) which made the error obvious.

In the opossum there is no differentiation of embryonic and trophoblastic cells. The entire vesicle corresponds to the ovum of a fish, an amphibian, a reptile, or a bird, and no line can ever be drawn between the cells which are to be retained in the definitive body and those which are to be discarded.

As mentioned in the first part of this chapter the unilaminar blastocyst shows no polarity or visible differentiation of any sort. Probably most of the cells at this time are indifferent protodermal cells—just as Mangold ('24) has shown most of the cells of the amphibian egg are at the beginning of gastrulation. But a few have a peculiarity [7] which makes them later enlarge and migrate to the interior. These constitute the endoderm and probably also part of the axial mesoderm, the prechordal plate, which though embedded in the endoderm may be seen proliferating mesoderm in stage 22. The 'invaginated' material then induces the formation of a medullary plate in the hitherto pluripotential external material.

These facts are perfectly parallel to what is known to occur in the lower vertebrates, and they make the primitive mammalian blastocyst fit naturally into the phylogenetic series.

[7] I do not mean, of course to assert that the peculiarity which causes the endodermal mother-cells to enlarge and migrate into the blastocoel will never be traced back to some sort of cytoplasmic localization in the uncleaved ovum. Nor do I mean that there is any a priori objection to the idea that such material, if existent, may be consistently allotted to one of the first to blastomeres. All I mean is that this peculiarity which causes some cells to migrate internally and subsequently to induce a medullary plate would correspond to the primary organizer, not to the whole embryo, and that we do not yet know whether the plane of the first cleavage bears any constant relation to any such material or not. In the only mammal for which experimental evidence is at present available (the rat), Nicholas ('34) has shown that either of the first two blastomeres can produce a whole embryo.

Stage 12

The beginning of expansion of the vesicle. During the formation of the endoderm mother-cells and the primitive endoderm (stages 10 to 12) the vesicle enlarges from 0.17 mm. to 0.34 mm. This expansion, which is rather sudden, is probably due for the most part to stretching under the influence of fluid accumulating in the blastocoel. It results in a noticeable thinning out of all the cells of the vesicle, though those of the medullary plate still appear thicker in sectional view than any of the others.

The eggs of Hartman's litter no. 194′ were removed from the uterus exactly 4 days after copulation, and form a good type series for stage 12.

VI. THE FIFTH AND SIXTH DAYS

Stages 13 and 14. The definitive endoderm. The bilaminar blastocyst. The uterine glands.

Stage 13

The definitive endoderm. In the early part of the fifth day the primitive endodermal cells begin to migrate beyond the bounds of the medullary plate, and as they do so they become very much flatter and exhibit long pseudopod-like processes. The migration is almost certainly accomplished by amoeboid movement, though this cannot be established beyond question with evidence from fixed material alone. These new, extremely flattened, migrating cells are the definitive endoderm.

About the time the endoderm begins to migrate beyond the medullary plate two changes in the gross aspect of the vesicle become apparent: 1) it changes from a perfect sphere to an ellipsoid with the shortest diameter in the direction of the egg axis; and 2) it becomes eccentric in relation to the egg shell and albumen (stage 13 and fig. 15).

The explanation of the first of these two changes is not clear, but it is fairly certain that the second, the eccentricity of the vesicle, is due to the more rapid absorption of the

albumen at the animal pole than at the vegetal. This difference in rate of absorption is so decided that it causes the vesicle to migrate across the albumen layer until the medullary plate comes into contact with the shell membrane. This

Fig. 15 Stage 13 ellipsoid blastocyst showing medullary plate in contact with shell membrane (Hartman, '19).

Fig. 16 Stage 14 bilaminar blastocyst (Hartman, '19).

contact is not attained until the end of the sixth day; and by this time the endoderm has extended itself completely around the inside of the vesicle.

During the rapid increase in diameter which accompanies the migration of the endoderm the walls of the vesicle become markedly thinner. This thinning affects all portions of the vesicle but in the medullary plate it seems less conspicuous. The migration of so much endoderm from beneath the medullary plate, however, reduces that region to a thickness of only two cell layers again (i.e., medullary plate and endoderm). The medullary plate remains distinctly thicker than any other portion of the vesicle.

The end of the fifth day is not characterized by any very distinctive feature. The vesicle has enlarged to about 0.50 mm., and has not yet reached the shell membrane. The vegetal pole is at the center of the albumen. The endoderm extends to the general neighborhood of the equator of the vesicle.

Stage 14

The bilaminar blastocyst. The end of the sixth day is marked both by the contact of the medullary plate with the shell membrane, and by the completion of the endodermal lining of the vesicle. The diameter of the vesicle at this time is about 0.75 mm. (stage 14 and fig. 16).

The cells of the different layers show the following distinguishing characteristics. The ectodermal cells outside the medullary plate are intermediate in thickness; they stain more lightly than any of the others; they contain practically no yolk granules; their nuclei are far apart (on account of the flatness of the cells); and their cytoplasm is rather easily broken down by fixing agents. The medullary plate cells are nearly columnar, so that their region is thicker than any other part of the vesicle and their nuclei are very close together; their cytoplasm is denser and more yolk laden than that of the rest of the ectoderm, and rather more basophilic. The endodermal cells are thinner than any others; their nuclei are larger in polar view than those of the ectoderm, and they stain very darkly.

As the animal pole at this time has absorbed all of the overlying albumen, it probably begins to derive nutriment from the so-called uterine milk—a secretion from the uterine glands to be described presently, which filters through the shell membrane. The vegetal pole is still covered by albumen, which it is absorbing slowly.

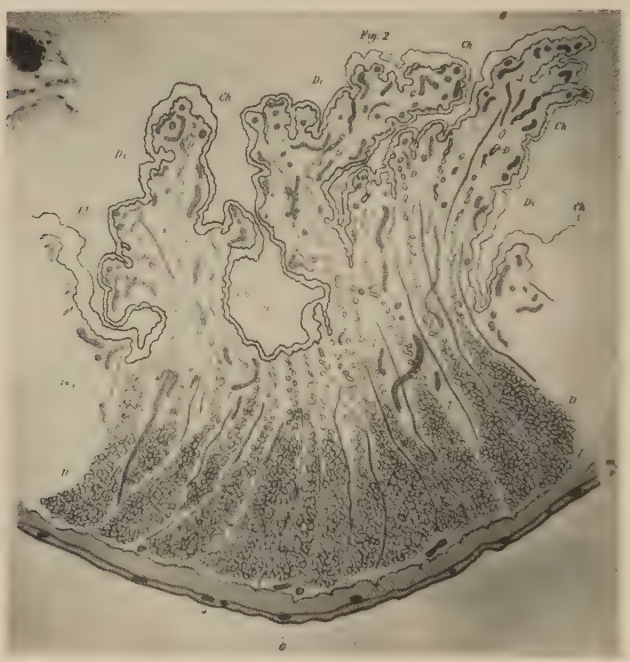


Fig. 17 Uterine glands (Selenka, 1887).

The uterine glands. Inasmuch as the uterine glands play no part in the development of the vesicles until the sixth day, I have postponed discussion of them until this point; but this arrangement necessitates a short digression now to summarize their previous history.

Selenka (1887) was the first to describe and figure the condition of these glands during pregnancy (fig. 17). Hartman ('23) traced their development throughout the oestrous cycle.

During the anoestrum (October, November, December) the uterine mucosa contains many long, tubular, fairly straight, simple, or double-branched glands. The walls of these glands are made up of low columnar or cuboidal cells with large nuclei that nearly fill the cell body. The lumen of the gland is small, and is not apparently ciliated. During the prooestrum (first week of January and the beginning of each oestrous cycle thereafter [vide, chapter 1]) the glands begin to lengthen and coil. The peripheral half of the thickness of the mucosa is the region where the coiling of the glands is most pronounced and conspicuous—so much so that the mucosa in sections appears to be made up of two layers. In the medial layer the glands are straighter and are separated by wide lymph spaces; in the distal layer they are so complexly coiled that they occupy most of the available space. At this time the nuclei, for the most part, migrate to the bases of the cells, and cilia become abundant in the lumen. During oestrus and ovulation the lumen of the gland enlarges, the number of mitoses increases, and the cilia become more prominent. During pregnancy the glands increase progressively in their complexity, even becoming considerably coiled in the neighborhood of the endometrial surface. All the nuclei are basal, and no cilia have been recognized.

The secretion of these glands is a clear, cell-free, lymph-like fluid which is delivered into the uterine cavity and absorbed by the vesicles. The animal pole, as we have just seen, is at the end of the sixth day the first part of the vesicle to exhaust the supply of the albumen above it, and must from this time forward depend upon this secretion for nourishment. The rest of the albumen supply disappears very shortly afterward and the entire vesicle is thereafter nourished only by the secretion of the uterine glands.

VII. THE SEVENTH DAY

Stages 14 to 17. The late bilaminar blastocyst. The primitive streak. The mesodermal crescent.

Stages 14 and 15

The late bilaminar blastocyst. In the first half of the seventh day the blastocyst enlarges from a diameter of 0.75 mm. to one of 1.0 mm. During this enlargement all of the ectoderm gains in thickness, but the endoderm remains as thin as it became during the fifth and sixth days. The medullary plate lies in immediate contact with the shell membrane. Its cells are quite crowded and are high-cuboidal in shape. Their cytoplasm is granular, and very chromophilic. All of these peculiarities make it a well-marked structure, conspicuous in whole mounts or sections. The rest of the ectoderm is somewhat less sensitive to injury by fixing agents than it was when it was thinner. It is in close contact with the small amount of albumen left between it and the shell. This albumen disappears as the vesicle enlarges at its expense, and consequently the vesicle gradually regains its spherical shape. The nuclei of the endoderm still appear larger than those of the ectoderm (possibly because they are flatter), and still stain very deeply. Both layers are in close contact at all points.

It should be mentioned that occasionally some endodermal cells in a restricted region near the margin of the medullary plate become noticeably larger than the others. The significance of this change is not definitely known. Hartman ('19, p. 86) suggests that they mark the region above which the primitive streak is soon to arise. It seems possible that they represent the prochordal plate, a portion of which, the prechordal plate, will later be seen proliferating mesoderm.

During the second half of the seventh day the last traces of albumen disappear. The vesicle is again perfectly spherical. When the diameter is about 1.4 mm. the medullary plate in a small circular area becomes thinner, so that by transmitted light this region appears as a light spot near one

edge of the relatively opaque disc (stage 15). This light spot, which was first described by Hartman, is the area in which the first mesodermal cells appear.

Stage 16

The primitive streak. When the vesicle is about 1.8 mm. in diameter Hartman discovered that certain cells in the peripheral half of a radial line through the center of the light spot mentioned above, drop down beneath the medullary plate and lie between the latter and the endoderm. These are the first mesodermal cells. When enough of them have collected in this region to form a slight local opacity they show up in transmitted light as a dark streak in the light spot at the point where the latter is nearest the edge of the medullary plate (stage 16). This is the primitive streak. It is the only part of the vesicle which is 3 cell layers thick.

It may seem strange on first thought to find the primitive streak arising within the medullary plate. The process of 'gastrulation,' or its equivalent, began back in the fourth day with the origin of the endoderm. The primitive streak stage, as is always the case, represents a late stage of gastrulation, and it is not surprising at a late stage to find the equivalent of the blastopore within the medullary plate. Such a situation is universal, and is, of course, the explanation of the neurenteric canal in those forms in which one occurs.

From this time (stage 16) right on through the end of the tenth day (stage 29) the primitive streak will be permanently recognizable in the posterior growing tip of the medullary plate—the sinus rhomboidalis. During stage 30, the first half of the eleventh day, the posterior neuropore will finally close over, and the primitive streak will be lost in the floor of the spinal cord of the tail.

The origin of the first mesodermal cells is the point at which Hartman's studies of the early embryology of the opossum came to an end. An exhaustive account of the origin of these cells, the formation of Hensen's node, and the spreading of the mesodermal crescent, is in preparation by Dr. C. H.

Heuser of the Carnegie Institution. His material, which is very complete, is that which was collected by himself and Doctor Hartman in 1918. They have kindly allowed me to take the figures for my stages 15 to 20 from some of their beautiful photographs. As Doctor Heuser's account of the next four stages will be much more detailed than I am able to give, I shall content myself here with listing the distinctive features which I have chosen as criteria for defining them.

Stage 17

The mesodermal crescent. Hartman's litters 346 and 346′ show the changes which occur between the middle and the end of the seventh day (interval of $9\frac{3}{4}$ hours). The eggs of 346 represent late stage 14, and those of 346′ are types for stage 17. The latter are 2.0 mm. blastocysts characterized by a strongly developed primitive streak from the posterior portion of which mesoderm streams laterally to form two symmetrical wings—the mesodermal crescent.

The remaining three neurula stages (i.e., stages with no sign of organogenesis except the medullary plate) belong to the eighth day, and are described in the next chapter.

VIII. THE EIGHTH DAY

Stages 18 to 23. Hensen's node and the primitive groove. Elongation of the medullary plate. The mesodermal rim. The notochord and the medullary groove. The first somites and the parietal mesoderm. The coelomic cavities and the subcephalic fold.

Stage 18

Hensen's node and the primitive groove. In the early part of the eighth day when the vesicle has attained a diameter of 2.2 mm. a distinct thickening of the external layer appears at the anterior end of the primitive streak. This is Hensen's node. At this point ectoderm and endoderm seem to fuse, and mesoderm may be seen proliferating

from this fusion in a lateral direction, as well as from the whole length of the primitive streak. The latter structure has developed two folds along its edges near the posterior tip so that it may now more appropriately be called the primitive groove. The mesoderm occupies only a small oval region around the primitive groove, and beyond this region the medullary plate is still in contact with the endoderm. Hartman's litter no. 338 represents this stage.

Stage 19

Elongation of the medullary plate. Shortly after the primitive streak appears the posterior portion of the medullary plate including it begins to grow in a caudal direction. This process actually begins in stage 18, but it becomes conspicuous for the first time in stage 19. At this time the whole primitive streak has become converted into a groove, and its edges have begun to wrinkle as if they are growing too fast for the surrounding cells to keep up with them. This is particularly true at the posterior tip. The mesoderm now extends anterior to Hensen's node, but there remains a crescentic strip of the anterior portion of the medullary plate which is still in contact with the endoderm. It is interesting that at this time the mesoderm is continuous across the midline in front of Hensen's node.

Stage 20

The mesodermal rim. Just before the mesoderm reaches the anterior extremity of the medullary plate it begins to extend beyond it posteriorly. The extreme edge of this spreading sheet forms a dark irregularly circular line—the mesodermal rim—along which the mesoderm is fused with and possibly arising from the endoderm. In stage 20 this line first becomes visible beyond the medullary plate in the posterior region. In slightly later specimens (e.g., 17179) it has migrated well beyond the medullary plate and completely surrounds it.

Being the border of what will later become the area vasculosa, the prospective site of origin of the sinus terminalis, and a region of fusion of mesoderm and endoderm, the mesodermal rim has much in common with the 'germ wall' of avian eggs. The absence of yolk in the opossum vesicle at this time, however, makes the question of their homology difficult to decide.

At this stage the primitive groove attains its greatest relative and absolute length, and simultaneously a clear spot appears at its anterior tip. The vesicle is now 3.2 mm. in diameter.

Stage 21

The notochord and the medullary groove. The clear spot in stage 20 is due to the shortening of the primitive streak at its anterior end. When the vesicle is about 3.7 mm. in diameter (specimen 17179) this shortening at the anterior end is going on more rapidly than the growth at the posterior end, so that the primitive streak is decreasing in total length. The shortening process is correlated with the appearance of two new structures—the notochord and the medullary groove—for it seems to be due to a tangential split in the substance of Hensen's node. The lower or more medial cells are separated from the more superficial cells and become the notochord. This leaves only a thin sheet of medullary plate in the middle line above the notochord, which, correspondingly, when illuminated from below appears as a light streak. The medullary plate sinks in slightly along this thin line and thus forms a medullary groove which is anterior to the primitive groove.

During all this time the medullary plate is constantly increasing in length. Most of this increase seems to be due to the multiplication of cells at the posterior growing tip; but part of it may be due to migration of cells, for the plate is also narrowing in width at the same time that it is increasing in length and I see no indication of changes in cell shape which might account for this.

Stage 22

The first somites and the parietal mesoderm. In specimen 17180 (see stage 22 in the second normal stage plate)in which the medullary groove has become nearly twice as long as the primitive groove, two slight condensations of the mesoderm beneath the medullary plate on either side of the central mesoderm-free strip represent the first somites.

Selenka figured (somewhat diagrammatically) a similar specimen (1887, Taf. XX) with three somites. In it he recognized a longitudinal mesodermal thickening lateral to the medullary plate, which he called the parietal mesoderm. In the absence of a series of intermediate stages, however, he did not realize that the ectodermal thickening which he now correctly calls the 'Medullarplatte' is the same ectodermal thickening which in the bilaminar blastocyst he labeled 'Keimscheibe.'

This ectodermal plate, which first appears in stage 11 (latter part of the fourth day), is continuously and conspicuously recognizable from then on. Its visible distinction from the rest of the vesicle in all the early stages seems to be due principally to a greater density of its cytoplasm. In fixed but unstained specimens this dense plate is more opaque than the rest of the vesicle, so that it appears darker by transmitted light and lighter by reflected light. In stained specimens it is always more chromophilic—not particularly basophilic or acidophilic, but more retentive than the rest of the vesicle for almost all stains.

I emphasize this difference in density of cytoplasm and consequent staining reaction because the distinctive feature usually attributed to this plate (that of thickness) is rather inconsistent. At the time when the vesicle is swelling rapidly under the pressure of fluid accumulating in the blastocoel (stages 11 and 12) the less dense portion of the vesicle becomes enormously stretched and flattened, whereas the denser medullary plate remains comparatively thick and occupies a smaller proportion of the surface. Later on, the so-called

trophoblast thickens again, and many of Hartman's own figures ('19, pl. 22, figs. 1, 2, 4, 4A, 3B, 5, 6, 8, 9) show practically no differentiation between trophoblast and 'embryonic area' except that of staining reaction. In his figures 2 and 4 the trophoblast at the opposite pole is decidedly thicker than the 'embryonic area.' Selenka (Taf. XIX, fig. 6) shows the same thing. In still later stages (stages 20, 21, and thereafter) the trophoblast at the opposite pole becomes at least twice as thick as the medullary plate, but the medullary plate is thicker than the immediately adjoining non-medullary ectoderm and much more dense than the opposite trophoblast.

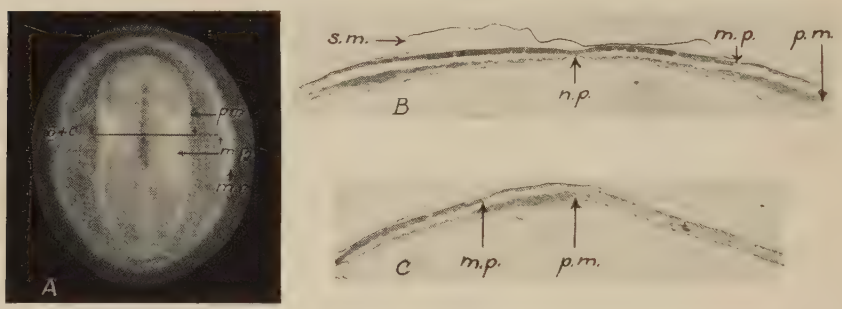


Fig. 18 Stage 22. A, photograph of whole mount 16139. B, photograph of section of same specimen showing medullary plate, notochordal plate, parietal mesoderm, and shell membrane. C, more lateral view of same section.

There is thus no stage after its first appearance when this ectodermal plate is not sharply demarcated in sectioned specimens by density, staining reaction, thickness, or all three, from the rest of the vesicle. In external views (see the second normal stage plate) it is easy to recognize until stage 22.

Figure 18 A is a photograph of specimen 16139 (stage 22) before it was sectioned. The long transverse line indicates the level at which the section represented in 18 B and 18 C was taken. The two arrows above the line show the extent of the section photographed in 18 B, and those below the line show the extent of 18 C. Figure 18 B shows clearly that the parietal mesoderm is lateral to the thick ectodermal plate.

Figure 18 C, a more lateral view of the same section, shows that the ectoderm covering the parietal mesoderm is continuous with and indistinguishable from the extra-embryonic ectoderm, which means that there is no such thing as an embryonic area in the sense in which this term is ordinarily used in mammalian embryology. The opaque light edge of the 'embryonic area' seen in the whole mount (fig. 18 A, p.m.) is the edge of the mesodermal thickening, not the ectodermal thickening. This could not be determined from an external view, but in sections it is obvious. After this stage it is easy to see even in external views that the ectodermal thickening is a medullary plate. The hearts, the limb ridge, and the whole body wall form lateral to it.

In describing his 3-somite specimen Selenka says (S. 125):

Was aber den peripherischen Endsaum den "Keimwulst" des Mesoderms betrifft, so zeigte sich hier nirgends die mindeste Andeutung einer Entstehung von Mesodermzellen in loco, noch eine Betheiligung des Ektoderms oder Entoderms; er markirte sich auf den Schnitten niemals als Wulst oder Anschwellung, und erst mit der Anlage des Sinus terminalis erscheint auch die peripherische Grenze des Mesoderms bezw. des Gefässblattes, scharf abgesetzt.

As pointed out in a previous paragraph the edge of the spreading sheet of mesoderm is always sharply defined from the very beginning, and the mesoderm definitely merges with the endoderm along this line. This line is the mesodermal rim, marked m.r. in figure 18 A. Whether any mesodermal cells are actually arising from the endoderm is difficult to determine, but at any rate endoderm and mesoderm are completely fused along this line, and many mitoses are found in the region of fusion.

Stage 23

The coelomic cavities and the subcephalic fold. I have only one specimen upon which to base a description of stage 23. This specimen (17181) was a retarded member of a litter, the other members of which belonged to stage 24. Also it

was very badly handled before sectioning. During an incautiously rapid dehydration the vesicle collapsed so that the sections are wrinkled and in many cases broken. In all of these repects the material is unsatisfactory, but as no specimen of this stage has been described before, and as no other material is available at present, it seems worthwhile to include it tentatively until better material can be obtained.

Fortunately the drawing of the external view was made before the vesicle collapsed. This is shown as stage 23 in the second normal stage plate. It can be seen that there are four well-formed somites. They lie beneath the medullary plate which is now easily recognizable even in a whole mount. Lateral to the medullary plate the parietal mesoderm forms a dark shadow. Anteriorly (i.e., in the region cranial to the somites) the parietal mesoderm has split to form the two coelomic cavities, which at this time do not extend beyond the pericardial region. There are no heart tubes recognizable in this specimen though the splanchnopleure is very decidedly thickened throughout the pericardial region, and must have been prepared to fold into myocardial tubes very soon.

No sensory anlagen are recognizable. The ectoderm is beginning to fold under the anterior edge of the medullary plate (the subcephalic fold), but the mesoderm is still continuous across the midline in front of the embryo so that there is no pro-amnion.

In the posterior fifth of the medullary plate the primitive groove is well marked. Immediately caudal to it and outside the medullary plate a light mark represents the spot where the ectoderm folds in slightly to form the anal pit or proctodeum.

IX. THE NINTH DAY. Stages 24 to 26

The first third of the ninth day

Stage 24. The heart tubes and the first blood vessels. The first sensory anlagen. The nephrogenic ridge. The first branchial pouches. The hypophyseal plate and the pharyngeal membrane. The proamnion. Differentiation in the

medullary plate and the neural crest. Miscellaneous details. Comparative notes.

The heart tubes and the first blood vessels. In two embryos which by somite number and general appearance should belong to my stage 24 Selenka was unable to find any blood vessels, though he described the hearts with their endothelial tubes. As he was also unable to recognize sensory anlagen in this material, it must be assumed that fixation was bad, or that many of the sections were lost, or that some other such accident made the description of the internal anatomy of these specimens incomplete.

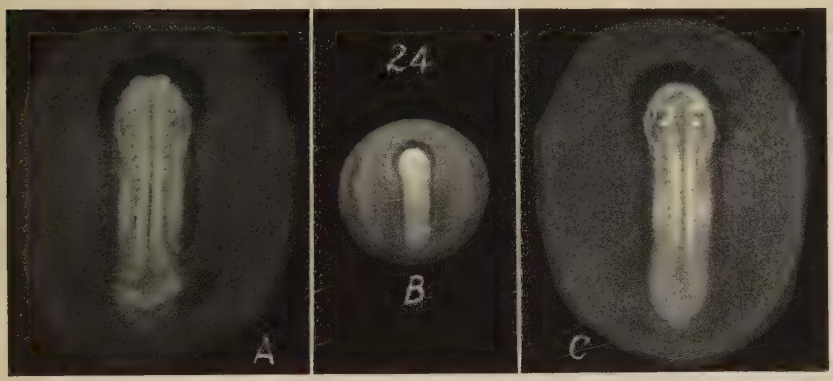


Fig. 19 Photographs of external views of stage 24. A, dorsal view of embryo and area vasculosa. B, dorsal view of whole vesicle. C, ventral view of embryo and area vasculosa.

In the six sectioned specimens in The Wistar Institute collection which belong to this stage there is a considerable vascular system. The hearts contain endothelial tubes which communicate anteriorly with a capillary plexus running through the mandibular mesenchyme, and posteriorly with a vitelline plexus from the yolk sac (fig. 20, totomount and section L). The mandibular plexus on each side connects medially with a dorsal aorta, also somewhat plexiform in early specimens, which runs caudally beneath the medullary plate. At the level of the first few somites the two aortae are

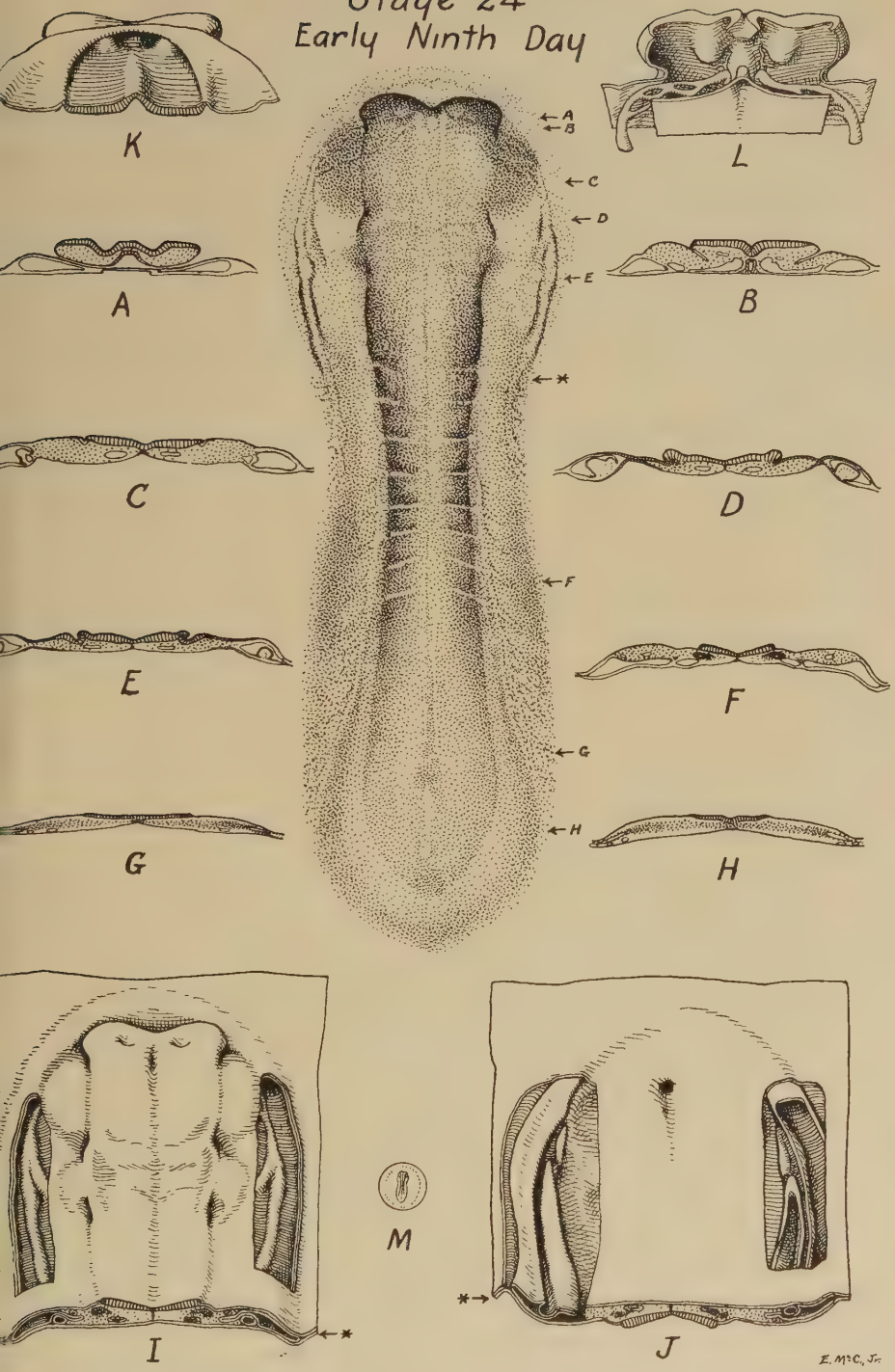
gradually diverging laterad to pass from beneath the medullary plate at about the level of the fourth somite. Beyond this point they give off a few small intersomitic twigs, from which branches pass to the limb ridge, and finally they break up into a complicated plexus leading ventrally and laterally into the area vasculosa.

In connection with the hearts a most unexpected detail is represented in the totomount drawing (fig. 20) between the levels indicated by the arrows D and E. Here may be seen a large vessel branching from what appears to be the heart tube medially toward a slight mesenchymal opacity at the edge of the medullary plate. This vessel turns out to be the primordium of the second aortic arch, and its point of junction with the lateral tube marks the anterior extremity of the heart tube proper. The mesenchymal condensation is the hyoid mesenchyme. In the late specimens of stage 24 the head region is rounding up so that the vessel, which ran medially at first, comes to run dorsally into the hyoid arch. At this time there is no corresponding dorsal twig from the aorta, but such a twig develops in stage 25. Then the capillary plexus and every stage in the differentiation of the arch can be followed in stages 26 and 27.

Four veins appear during stage 24. The first, the vitelline (or omphalomesenteric), is the vein of the yolk sac. This will be by far the most important vein in the embryo until just before birth. It grows out as a capillary plexus from the posterior end of the heart tube, which meets a similar plexus arising in the area vasculosa and growing toward the embryo.

The next two veins, the vena capitis medialis (or primary head vein) and the allantoic (or umbilical) arise simultaneously. Many small vessels which appear to be remnants of the same primitive capillary plexus from which the aortae

Fig. 20 Totomount, sections, and reconstructions of stage 24. Totomount—Edinger apparatus drawing from 17176 with arrows to indicate levels of sectional views. The level marked with the asterisk is shown in sectional view in I and J. All sectional views and reconstructions are from 17153. M indicates the actual size of vesicle, embryo, and area vasculosa.


Stage 24
Early Ninth Day
K
L
A
B
C
D
E
F
G
H
I
M
J
E. McC., Jr.

arose, run together in the mesenchyme under the midbrain and grow back between the otic placode and the medullary plate. They pass the second branchial pouch (which will be described below) on its medial side (later dorsal) and end blindly except for scattered capillary communications with the dorsal aorta. These vessels constitute the anlagen of the primary head vein.

At the same time a similar plexus appears in the parietal mesenchyme dorsal to the point where the vitelline vein enters the body. From this plexus one channel eventually becomes enlarged. It lies (fig. 20, I) close against the parietal pericardium, which in turn is in contact with the visceral pericardium at this point, but no communication is attained until late stage 25. From this level it grows caudad until its posterior tip reaches the limb ridge (fig. 20, F). This vein, the umbilical, is thus in early stages exclusively a vessel of the anterior limb and the lateral body wall, and in the opossum this primary association is preserved until a relatively late stage of development (stage 30, see p. 165).

In the latest specimens of stage 24 (e.g., 17136, 12 somites) a continuous border vessel is formed around the area vasculosa—the sinus terminalis. It has innumerable communications with the yolk sac vessels and through them eventually with the vitelline and aortic plexuses from the embryo.

The first sensory anlagen. In stage 23 no sensory anlagen were found, though it should be remembered that my material for that stage is inadequate. In the earliest specimen of stage 24 which I have (17153) both the optic cups and the otic placodes are present, so that I cannot establish any priority between these two.

As far as my evidence goes the optic cups are paired and lateral from the beginning (fig. 20, totomount, A, I, K, L). At first they are exceedingly difficult to recognize in totomounts unless the lighting is carefully adjusted. But they show very clearly in the photograph (fig. 19, A) and I do not doubt that if Selenka's light had been satisfactory he would have found them in the specimen he figured in his

Tafel XXI, and they were very likely also present in his slightly younger specimen shown in Tafel XX. In sections, of course, there is not the slightest difficulty in identifying them (fig. 20, A).

The acoustic placode (fig. 21) is situated on the dorso-caudal surface of the hyoid swelling just anterior to the second branchial groove (v.i.). This section (17153, 13,4,12) passes through the anterior portion of the placode, which is flat, but figure 20, E, shows that posteriorly the placode wrinkles under the edge of the medullary plate.

For the discussion of the sensory ganglia see the section on the medullary plate and the neural crest.

The nephrogenic ridge. The pronephros in the opossum is no better developed than in other mammals, and no less

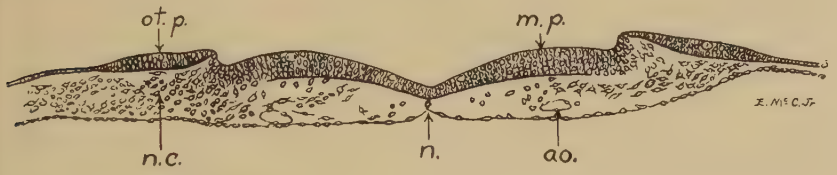


Fig. 21 Section through otic placodes and medullary plate at stage 24 (17153, 13, 4, 12).

well developed than in the lower amniotes. At the height of its development it is composed of only a few very small, solid tubule anlagen attached at one end to the coelomic epithelium and at the other end to a solid duct. As the tubules are solid no true nephrostomes appear, though in many cases a small indentation in the coelomic epithelium at the point of origin of the tubule may represent the vestige of one. The development of the pronephros, however, has acquired a renewed interest in the light of the theory recently proposed by Burlend ('31), which will be discussed below; and the details of this development in the opossum turn out to be significant in connection with this theory.

Of the six specimens representing stage 24 in The Wistar Institute collection, all but one show definite signs of nephrogenesis. The one exceptional embryo (17166) is abnormal in

several other respects (distended coelomic cavities, highly elevated embryonic region, excessively dense mesenchyme with retarded vascularization) and so probably should be omitted from this discussion.

The most anterior pronephric anlage is always found opposite the seventh somite. The intermediate mesoderm is unsegmented and, at this level, very sparse. In the earliest specimen (17153) a distinct condensation involving only a few cells is formed in the intermediate mesoderm immediately adjoining and continuous with the somatic layer of the lateral mesoderm. This condensation appears in individual sections as a knob projecting medially from the coelomic lining, but in series it shows itself to be for the most part a ridge. The ridge tends to be thick and round on its free edge (where it is broken away from the somites or dorsal mesoderm) and quite thin on its attached edge, which latter occasionally breaks through, leaving the thicker edge as a cord of cells running parallel to the coelomic epithelium. These breaks, however, are small, irregular, far apart, and quite unrelated to the segmentation of the dorsal mesoderm.

Beyond the last somite (the tenth) the intermediate mesoderm becomes much more abundant, is still markedly condensed, but not completely separated from the dorsal unsegmented mesoderm, and also not from the lateral mesoderm. This is the condition back to the neighborhood of the primitive streak where dorsal and intermediate mesoderm become indistinguishable.

There is thus a continuous condensation of the intermediate mesoderm from the level of the seventh somite to the posterior end of the body, which condensation shows a tendency to separate from the dorsal segmented mesoderm and remain as a ridge on the coelomic lining. In the next specimen to be described this nephogenic ridge will be seen to be the anlage of the wolffian duct and of all the tubules of the pronephros and mesonephros.

Specimen 17139 represents the end of stage 24. Figure 22 shows a reconstruction of the nephrogenic ridge in this specimen from its anterior extremity to the end of the eleventh

somite. This includes all of the pronephric region and the beginning of the mesonephric.

The pronephric anlage extends from a point opposite the middle of the seventh somite to a point opposite the middle of the tenth. There are no separate tubules. The thick, rounded edge of the ridge, which represents the pronephric duct, is continuous antero-posteriorly, and is connected with the coelomic lining laterally by a thinner edge from which the tubules will be differentiated. What breaks there are in the tubular portion at this time bear no relation to the somites, but occur at both somitic and inter-somitic levels. The well-formed break near the beginning of the tenth somite admits the passage of a small artery from the dorsal aorta.

Fig. 22 Reconstruction of nephrogenic ridge in 17139. The somite levels are indicated above the reconstruction. The dotted ellipses near the right end of the figure indicate the extent of the lumina of the anlagen of the first four mesonephric tubules.

Near the beginning of the eleventh somite the thick portion of the ridge enlarges very markedly, the dorso-ventral diameter (that perpendicular to the plane of the paper) being particularly increased. This marks the beginning of the mesonephros. In section the more dorsal portion can be recognized as the anlage of the wolffian duct, which does not yet possess a lumen. The more ventral portion already shows some organization into tubules, the lumina of the first four of which are indicated in the reconstruction by dotted lines. But the tubule anlagen have not become detached from each other, or from the somatopleure, or from the wolffian duct. In fact, in the opossum the mesonephric tubules are not at any time separate from the wolffian duct. They are formed from the same anlage. Only the lumina of the duct and the tubules arise independently and later coalesce. The walls of

the duct and the walls of the tubules are continuous from the very beginning.

The important features of this development from a theoretical point of view are: that pronephric tubules, mesonephric tubules, and wolffian duct all arise from the same anlage; that this anlage is a longitudinal unsegmented structure which is continuous from its anterior end back to the region of the prospective cloaca; and that this anlage has for awhile the form of a ridge on the coelomic lining.

All of these features are in accord with Burlend's theory, which is, in part, that "the nephric system originated phylogenetically from a right and left 'primitive nephric groove' which became differentiated from the somatopleuric mesoderm lining the primitive splanchnocoel;" that "the groove became closed off from the splanchnocoel at intervals, and thus a duct . . . . was evolved." This is to be contrasted with the familiar textbook account, which may be called the Brauer-Rabl theory, according to which the tubules arise first as separate segmental units. Their distal tips then grow together to form a duct (Sammelgang) which from the posterior end of the pronephric region sprouts independently (Endabschnitt) all the way back to the cloaca.

There is much evidence that the Brauer-Rabl theory is based upon some special cases (principally that of Hypogeophis) in which the intermediate mesoderm becomes segmented before nephrogenesis begins. Burlend was able to adduce strong contradictory evidence from cyclostomes, elasmobranchs, teleosts, ganoids, and dipnoi, all of which groups are more primitive than Hypogeophis and should accordingly be more significant. The pronephros in all the amniotes is so vestigial that not much hope has been entertained of securing interesting evidence from this source. The opossum evidence, supporting as it does the Burlend theory, is of particular interest, therefore, because it comes from the highest group of amniotes—the mammals.

One implication of the theory which is of general interest should be mentioned here. If the theory of the non-metameric

origin of the nephric system should prove to be the correct one, as the present writer believes it will, the last substantial evidence for either the annelid or arthropod theory of the evolution of the chordates will have been removed. For every other structure in the chordate body which exhibits any degree of metamerism can be shown to arise in the first instance as a non-metameric structure. The medullary plate, the neural crest, the mesoderm and its coelom, the notochord, the chondrostyle, and other structures to be mentioned below, all acquire whatever degree of metamerism they possess secondarily. Even the coelomic pouches of Amphioxus arise from a longitudinal groove in the enteron. All this seems to mean that the chordates arose from unsegmented ancestors and acquired a certain degree of metamerism independently during their own evolution.

The first branchial pouches. At stages 14 and 15 (second normal stage plate) ectoderm and endoderm are in immediate contact throughout the vesicle. At stage 16 the first mesodermal cells begin to separate them. By stage 20 the mesoderm completely underlies the medullary plate and is beginning to extend beyond it posteriorly. In stages 21, 22 and 23 a wide vascular area is formed and the sheet of mesoderm is uninterrupted throughout this region except in the midsagittal line where the notochordal plate in the endoderm is in immediate contact with the keel of the medullary plate (fig. 18, B). Now at stage 24 migration or resorption of mesoderm at six different points allows ectoderm and endoderm to make secondary contact with each other.

Four of the six points of secondary contact constitute the closing plates of the first two pairs of gill pouches (McCrady, '36). These are best illustrated in figure 31 where under the caption, first branchial pouches, stage 24, ectoderm and endoderm are seen to form a broad plate of contact medial to the hearts and lateral to the medullary plate. I do not know of any other animal in which branchial pouches have been recognized before the pharynx has acquired a floor, but that these are actually the branchial pouches is obvious enough from the

uninterrupted series at The Wistar Institute showing their transition to the more familiar condition.

In external views by transmitted light these thin closing plates appear as very light areas as shown at the level D in the totomount and section in figure 20. In this particular series (17153) the second branchial pouches have not quite met the ectoderm (fig. 20, E). This illustrates the cephalocaudal progression in the formation of the pouches, which is shown in further detail in figure 31. More details of the origin and development of these pouches will be discussed in connection with the lung anlagen in stages 25, 26 and 27.

The hypophyseal plate and the pharyngeal membrane. As the subcephalic wrinkle, first observed in stage 23, pushes farther beneath the medullary plate it makes two contacts which are of permanent significance. The first is a contact of ectoderm with ectoderm, the ectoderm of the wrinkle with the infundibular depression in the medullary plate. The infundibular depression is seen from a dorsal view in figure 20, I, from a ventroposterior view in K, and from a ventroanterior view in L. In the last figure particularly, it is easy to see how the ectoderm in turning back upon itself in the midline will come to touch first the infundibular depression, and second, the minute endodermal diverticulum which is the first trace of the foregut. The plate which touches the infundibular depression will later form Rathke's pouch and the anterior lobe of the pituitary. That which touches the endoderm forms the pharyngeal membrane which separates the stomodeum or buccal pouch from the pharynx until stage 29 when it becomes resorbed.

Proamnion. The sixth point of secondary contact of ectoderm and endoderm mentioned above is the so-called proamnion—a crescentic region extending around the cranial part of the embryo as far back as the vitelline plexus. It appears in reflected light as a dark region (fig. 19, A) surrounding the head. This structure, which is identical with that seen in an $8\frac{1}{2}$-day, 3.4-mm. rabbit, or in a 4-somite, 23-hour chick, and

which in these forms is transitory, is in the opossum permanent; and, as Selenka has shown, it contributes the major part of the definitive amnion.

Differentiation in the medullary plate and the neural crest. The medullary plate has elongated until it is about six and one-half times the length of the primitive groove, which is still clearly visible in its caudal tip (fig. 20, totomount and section H); and in the brain region the anlagen of the three primary divisions are now distinguishable.

The forebrain consists only of its diencephalic portion, and is the region under which the subcephalic fold extends in figure 20 (totomount and I). It includes the above-mentioned optic cups and infundibular groove, and it is broader than the midbrain region next to be described.

The midbrain is demarcated laterally by two very shallow grooves best seen in sections (vide fig. 20, C). Anteriorly and posteriorly it is demarcated by the flaring edges of the wider fore- and hind-brains. Selenka was unable to see any definite lateral border to the midbrain. This is another indication that most of his information about this stage came from his study of the whole embryo before sectioning, and that some of the sections were either lost or injured. The groove which bounds the midbrain laterally being shallow and being underlaid by the thick opaque mass of mandibular mesenchyme is almost impossible to see except in sections.

The hind brain extends from the point where the gasserian ganglion (v.i.) is proliferating, back to the somite region. In its anterior third it includes at least three recognizable neuromeres (fig. 20, I). It will later show six.

The cordal portion of the medullary plate is composed of a narrower cervical region and a broader thoracic region. The latter, which is posterior to the seventh somite, includes Hensen's node and the primitive groove.

The neural crest is proliferating most abundantly just anterior and just posterior to the first gill pouch. These proliferations are at the sites of the anlagen of the gasserian and acoustico-facial ganglia, respectively (vide fig. 21), but

several facts seem to indicate that only a few of these cells actually contribute to the ganglia. In the first place the cells thus derived are perfectly continuous with the mesenchyme of the whole adjoining branchial arch. It is not until stages 25 and 26 that the ganglionic rudiments become fairly distinct from the mesenchyme. In the second place, when the ganglia do become distinct they are composed of a relatively small number of cells—much smaller than the total number which must have been proliferated during stage 24. And finally, there is the fact that until this proliferation begins there is no recognizable mandibular or hyoid mesenchymatous mass. There is mesenchyme in the head region from stage 20 on, but only a thin and even layer. Simultaneously with the proliferation of neural crest in stage 24 the mesenchyme becomes so abundant anterior and posterior to the first branchial pouch that it shows up in transmitted light as two dark masses (fig. 20, totomount), which thereafter may be followed through later stages as the mandibular and hyoid arches.

It seems necessary to believe, therefore, that a considerable portion of the mesenchyme of these branchial arches is derived from mesectoderm proliferated at the sites of origin of the gasserian ganglion and the acoustico-facial complex. It should also be mentioned that the visible evidence in the opossum does not support the view that the otic placode contributes any cells to the acoustico-facial complex, but in the light of experimental evidence from Amblystoma this visible evidence must at least be held inconclusive.

Posterior to the second branchial pouch the neural crest is again fairly abundant at the site of origin of the glossopharyngeal ganglion and the third branchial arch. In fact, in figure 19 a third opacity is quite distinctly recognizable in all three photographs.

In the cordal region of 17153 (early stage 24) there is very little neural crest proliferation, but in later specimens as 17166 and 17139 it is well formed. A consistent difference between the neural crest in the cranial and spinal regions seems to be that the former arises from a broad region extending a

little way down the wall of the brain plate, whereas, the latter arises from a more restricted region at the very edge of the medullary plate.

Miscellaneous details. No nephrocoele ever develops in the opossum, so the myocoele is never connected with the splanchnocoele. Also the intermediate mesoderm does not become segmented. The somites separate first from each other (i.e., anteriorly and posteriorly), then from the intermediate mesoderm (i.e., laterally). They then have the form of flattened rectangular sacs enclosing a slit-like myocoele.

The paired coelomic rudiments (i.e., splanchnocoeles) elongate during stage 24 until they meet anteriorly and posteriorly, thus surrounding the embryo. The anterior loop is seen in figure 20, I, J and A. It extends beneath the subcephalic wrinkle as the sectional view shows. However, instead of expanding farther forward and thus eliminating the proamnion, as is the case in other animals, in the opossum as the head grows forward and the subcephalic wrinkle grows deeper, this anterior loop of coelom is brought beneath the body and in stage 25 it is simply incorporated into the definitive pericardium, there being no extra-embryonic coelom anterior to the embryo thereafter. This has an important effect upon the constitution of the amnion, which will be discussed in chapter X.

The posterior loop of coelom remains largely extra-embryonic. The anal plate dips in between it and the medullary plate. Subsequently, as the caudal part of the body rounds up, the proximal part of the extra-embryonic coelom becomes ventral, enlarges, and receives the allantoic diverticulum as that develops in stage 28 (fig. 37 and fig. 39, L). The extra-embryonic somatopleure goes into the formation of the caudal amniotic fold, and the splanchnopleure serves as the bridge along which the vitelline artery passes to the area vasculosa.

The notochord in stage 23 was a broad plate in the endoderm except at its most posterior end where it was separate from the endoderm and proliferating from Hensen's node.

In stage 24 its relation to the endoderm has not changed, but it is no longer a broad plate except at its anterior end where it merges into the prechordal plate, and its posterior end where it lifts above the endoderm and merges into Hensen's node. In early specimens of stage 24 it is throughout most of its middle portion only one or two cells in thickness, the cells being tall, columnar, and extending from the endoderm to the medullary plate. In the totomount (fig. 20) its width at various levels can readily be observed, the narrowest points in this specimen (17176) being at the levels of the hind-brain and again between the sixth and seventh segments. This extreme attenuation, however, is peculiar to early specimens. In later ones like 17166 and 17138 the notochord is again a thick plate in the endoderm.

A mesodermal condensation at the level F in figure 21 is the earliest primordium of the forelimb. I know of no other case in which the forelimb is begun at such an early stage.

Comparative notes. The stage 24 opossum embryo resembles a rabbit of the same age more than it does any other embryo. It is nearly identical with Minot's ('05) rabbit embryo no. 624 in the stage of development of the heart tubes, the aortae, the area vasculosa, the notochord, and the proamnion. It is further advanced in having more somites, having optic and otic anlagen, a forelimb blastema, branchial pouches, infundibular groove in contact with hypophyseal plate, and a pharyngeal membrane. It is less advanced in having no caudal amniotic fold. In this last respect it is intermediate between the mammalian type and the reptilian type as seen in Lacerta agilis (Keibel, '04). The early and rapid development of the first somites is, as will be seen in later chapters, to some extent peculiar to the opossum itself.

The chick of 4 to 6 somites (23 to 24 hours incubation) is similar in most respects, but has fewer somites and a medullary plate which is more advanced with respect to folding, though not with respect to differentiation.

The second third of the ninth day

Stage 25. First contact of the neural folds. Fusion of the hearts in the bulbar region. Origin of the sinus venosus. Origin of the pharyngeal floor. The lung anlagen. Miscellaneous details. Comparative notes.

First contact of the neural folds. When there are twelve or thirteen pairs of somites the neural folds come together and fuse in the region of the myelencephalon just caudal to the otocysts (fig. 23, A). Almost as soon as the edges fuse, they pinch off from the overlying ectoderm and form a tube

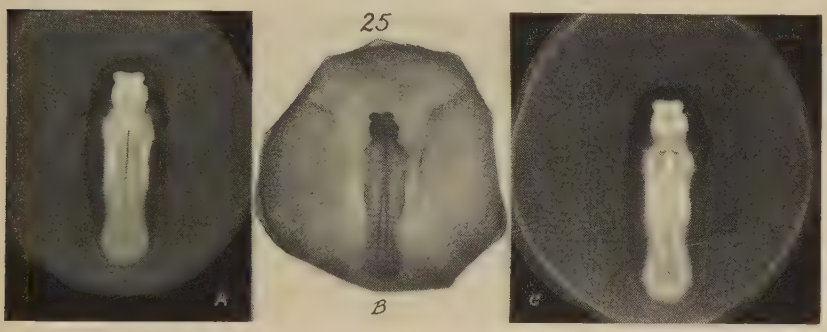


Fig. 23 Photographs of external views of stage 25. A, dorsal view of 16171 by reflected light. B, dorsal view of 16144 by transmitted light. C, ventral view of 16171 by reflected light.

which is laterally compressed (fig. 25, G and H). During the latter part of stage 25 the tube continues to close caudad through the first six or seven segments, that is, to about the anterior level of the limb ridge (fig. 23, B), but no progress at all is made craniad (fig. 25, A, B, C, D, E and F). In fact the brain plate will not begin to close over until the end of stage 27 (early tenth day, chapter X).

In the region of closure the neural crest seems to have ceased proliferating, and in the somite region it is aggregated segmentally. The gasserian ganglion and the acousticofacial complex (fig. 24) are rather well demarcated, but are still receiving proliferations from the brain plate. Neural crest

is also still proliferating from the edges of the posterior end of the neural plate.

Fusion of the hearts in the bulbar region. The process which leads to the fusion of the paired lateral hemicardia into the single median heart began when the pericardial sacs, which were completely separate in stage 23, grew forward around the head region and met in the midline in stage 24 (fig. 20, totomount, I and J). This region of first fusion of the pericardial sacs is anterior to both the aortic and cardiac primordia. The aortic plexuses lie for the most part outside the pericardia, but, as was shown in figure 20, J, the point of common origin of the first and second arches, and even a portion of each of these arches, is at first covered by

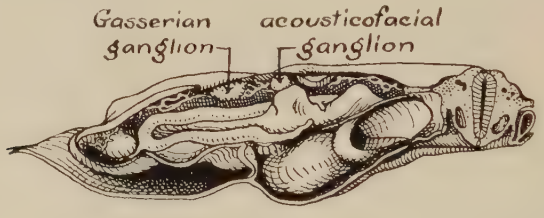


Fig. 24 Lateral view of reconstruction of stage 25 (17137).

myocardium. Accordingly, as the region of fusion of the pericardia progresses caudally the first portion of the endocardia to fuse is this bulbar portion. And the endothelial tubes which form the bases of the first aortic arches actually fuse before any part of the hearts proper. This is the condition in the last specimen of stage 24 (17139). In stage 25 (fig. 25, reconstruction and sections E and F) the fusion has not progressed beyond the bulbus cordis, but the ventricular portions of the tubes have been brought into close apposition. The reconstruction figure shows also that the atria are on either side of the anterior intestinal portal and are still widely separate.

The process of apposition and fusion of the pericardia and heart tubes in the opossum is somewhat peculiar in that it

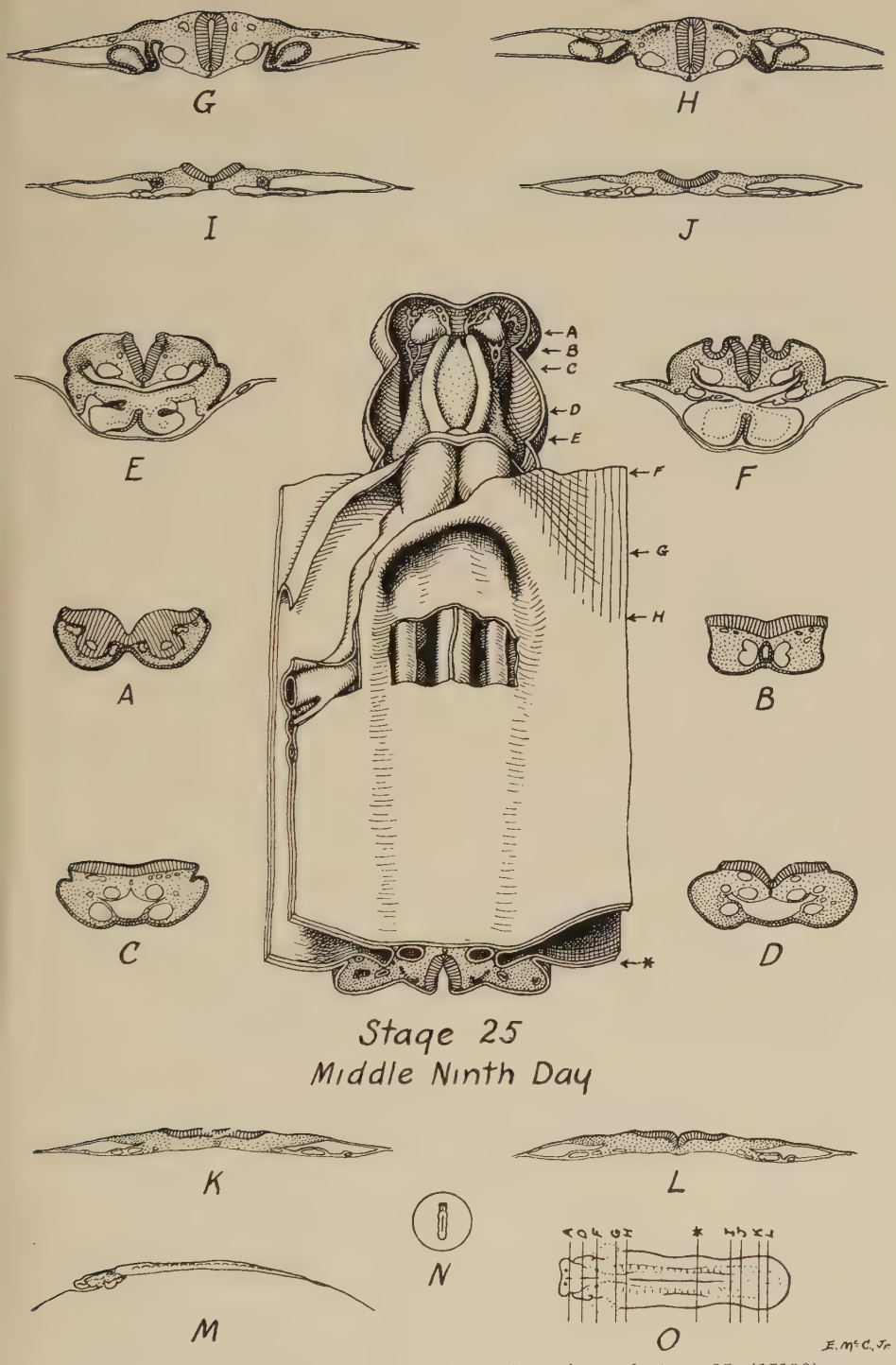


Fig. 25 Ventral view of reconstruction and sections of stage 25 (17136).

is accomplished without the formation of a ventral mesocardium at any time. This is illustrated in figure 26. The chick sections, taken from Patten's Early Embryology of the Chick, are presented for comparison. It will be seen that in

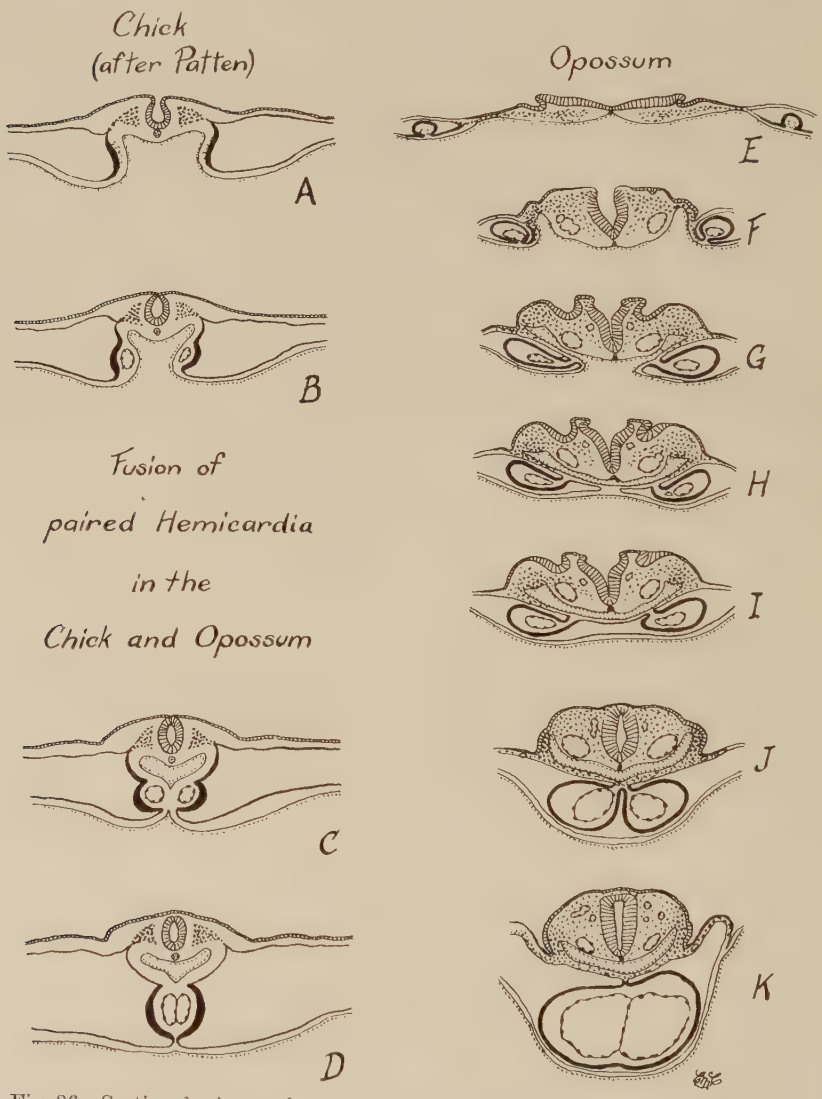


Fig. 26 Sectional views of opossum embryos of stages 24 to 26 and chick embryos (25 to 28 hours) to show that no ventral mesocardium ever forms in the opossum.

the chick the myocardial elements which approach each other near the midline are only half cylinders (B). As a result, when they fuse, mesenteries are formed both dorsal and ventral to the heart tube (C and D). In the opossum, on the other hand, each hemicardium is a nearly complete tube (E) long before the two approach each other. When they do draw near the midline (F, G, H, I) each is suspended by a single dorsal mesocardium. The two dorsal mesocardia fuse when the tubes meet (J and K), and no ventral mesocardium is formed.

Origin of the sinus venosus. In the last chapter it was mentioned that the allantoic vein arises just dorsad to the point where the vitelline vein enters the body. The two vessels are at first separated by a slight interval of coelom, but as they enlarge, the parietal layer of pericardium which underlies the allantoic vein, meets and fuses with the visceral layer which overlies the vitelline vein, and the vessels thus come into contact with one another (fig. 25, H). In late specimens of stage 25 their walls break down at the point of contact and they become confluent. Hereafter the original vitelline vein anterior to the point at which it receives the allantoic is the sinus venosus.

At the same time that this communication is established, capillary sprouts growing caudad from the vena capitis medialis, or primitive precardinal, reach this same level, and a few fuse with the allantoic vein near its entry into the sinus. The head vein thus gains access to the sinus. Some of the growing tips, however, pass this level (which is opposite the second somite) and continue caudad to the neighborhood of the fourth somite. These constitute the anlage of the postcardinal vein, which, however, will not be clearly established until stage 26.

Origin of the pharyngeal floor. In the early specimens of stage 24, though two branchial pouches with closing plates had formed, there was no pharyngeal floor beneath them. Figure 20, section B and reconstructions L and J, shows the small thimble-shaped diverticulum which is the anterior extremity and first-formed portion of the foregut. The same

process which leads to the concrescence of the heart tubes leads to the formation of a pharyngeal floor as seen in figure 27. By the end of stage 24 this process has extended the floor beneath the first branchial pouches (fig. 31), but the second branchial pouches are still over the empty yolk sac. During stage 25 the anterior intestinal portal moves caudad beneath them as the ventricles of the hemicardia draw near each other. The further migration of the portal caudad and some parallel changes in the shape and differentiation of the pharynx will be discussed in chapter XII in connection with the lung pouches.

Lung anlagen. Though the more familiar steps in the development of the lungs come after stage 27, the earliest primordia are distinguishable in late specimens of stage 24 and in all specimens of stage 25 (McCrady, '36). These primordia need special comment as they are different from any that have hitherto been described.

At stage 24 the endoderm in the cervical region shows on its ventral surface three longitudinal depressions: a medial notochordal groove, and two lateral branchial furrows. Each branchial furrow deepens locally at two points so as to extend through the flat body wall to the ectoderm. The points of contact between endoderm and ectoderm thus established have already been pointed out as the closing plates of the first and second branchial pouches. Posterior to them the furrow becomes shallower until it gradually disappears near the anterior limit of the pronephric anlagen. No such furrow ever develops in the endoderm of the thoracic, lumbar, and sacral regions.

At the level where the sections show the anterior end of the allantoic vein protruding through the somatopleure toward the vitelline vein, the pleuro-pericardial cavity is inserted between the lateral half of the branchial furrow and the ectoderm, and the splanchnopleuric mesoderm which thus comes into contact with the endodermal wall of the groove is conspicuously thickened. This thickening which is a center of rapid proliferation, is the mesodermal blastema of the lung.

The processes of formation of the branchial furrow, its deepening and subsequent development of local outpocketings show a definite cephalocaudal progression. This is best illustrated in figure 31 in which the principal point to be noticed at present is the striking similarity between the section through the lung region in stage 25 and that through the second branchial pouch in stage 24+. These two sections on casual examination might be confused. The only important differences are: first, that in the case of the lung anlagen the endodermal pouch does not actually touch the ectoderm; second, that the mesothelium in contact with the lateral side of the lung pouch is markedly thicker than that touching the branchial pouch; and third, that the location of the lung section may be identified by the presence of the anterior end of the allantoic vein immediately dorsal to and more or less in contact with the vitelline vein.

The endodermal constituent of the lung is thus a local outpocketing from a longitudinal groove, which outpocketing in early stages is indentical with others from the same groove which become gill pouches. The lung pouch is simply the hindermost gill pouch. Further evidence for this interpretation will be discussed in chapter X when the rudiments here described will be followed to a more familiar stage.

Miscellaneous details. The origin of the branchial pouches and lungs as secondary, metameric outpocketings from originally longitudinal, non-metameric grooves, is another illustration of the fact referred to above—that all metamerism in the vertebrates is secondary.

The notochord is in process of separation from the endoderm. From about the posterior end of the limb ridge back to Hensen's node it is a solid cord of cells quite free from the endoderm. But anteriorly it may be seen in all stages of pinching off. The originally flat plate wrinkles high in the midline so that its sides approach each other and include a narrow, vertical slit between them. When the sides unite ventrally, the notochord pinches off from the endoderm and sometimes shows a transitory notochordal canal; but where formed at all, this canal is quickly effaced.

The inner wall of the somites is breaking up and proliferating the sclerotome. The dermo-myotome remains as a square plate with its edges rounded inward.

The otic placodes have deepened into well-formed cups. The optic diverticula are becoming stalk-like.

The forelimb blastema has thickened into a very opaque plate.

Comparative notes. Rabbit embryos of 8½ to 9 days of age are at a comparable stage of development. In comparison with Minot's specimens 571, 573 and 621, the opossum at stage 25 is somewhat more advanced in differentiation of the hind-brain and cervical cord, the heart, the ear and the somites. And unless their anlagen have been overlooked in the rabbit, the opossum is also ahead with respect to the development of the lung pouches. The rabbit is ahead in having a distinct caudal amniotic fold.

The lizard of 1.5 mm. length (Peter, '04, N.T. 10) is at the same stage of development of the general body form, the central nervous system, the digestive tract, and the notochord. But the opossum is far ahead in the vascular and urinogenital systems, and in the eye and ear. The lizard is more advanced in the formation of a head amnion, but is like the opossum in still having no caudal amnion.

A 25-hour chick (7 somites) is like the opossum in central nervous system, digestive tract, vascular system, and in having no amnion of any sort. The opossum is much ahead of it in development of the urinogenital system.

Comment on the significance attributed to these comparisons is deferred to page 89.

The last third of the ninth day

Stage 26. Cephalic flexure. Beginning of amnion formation. Fusion of hearts in the ventricular region. Origin of the postcardinal vein. Miscellaneous details. Comparative notes. General comments on comparative data.

Toward the end of the ninth day the vesicles, which are still spherical and roll or float freely in the uterus (fig. 27, C), have attained a diameter of 9 or 10 mm. The embryo itself, however, is not more than about 6 mm. in greatest length, and it will not exceed this length until the end of the eleventh day. For between the ninth and the eleventh days several flexures occur, and the 'length' of the embryo undergoes such reduction and fluctuation that linear dimension loses all significance as a criterion of developmental stage. An embryo of 5.5 mm. greatest length may belong to stage 25, or to any of the

Fig. 27 Photographs of external views of stage 26. A, dorsal view of 16146. B, ventral view of 16145 after removal of non-vascular chorion. C, vesicles and part of uterine mucosa from Hartman's litter 291.

stages of the tenth day (27 to 29), or to the middle or late parts of the eleventh day (stage 31). After stage 31 crown-rump length becomes a useful datum for identification, and remains so until several weeks after birth when the head begins to assume a more axial alignment and it is necessary to shift to snout-anus measurements.

Cephalic flexure. The first of the flexures referred to is the so-called cephalic flexure, which is a right angle, ventral bend in the central nervous system at the level of the mesencephalon. It would be more proper to call this the mesencaphalic flexure, as the pontine flexure, which occurs in stage

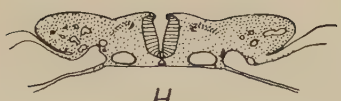
33, is equally 'cephalic;' however, the classical nomenclature here is probably too well established to be displaced.

This flexure in the opossum occurs at a time when closure of the neural folds has not progressed anterior to the myelencephalon (fig. 28, reconstruction and sections A, B, C, D).

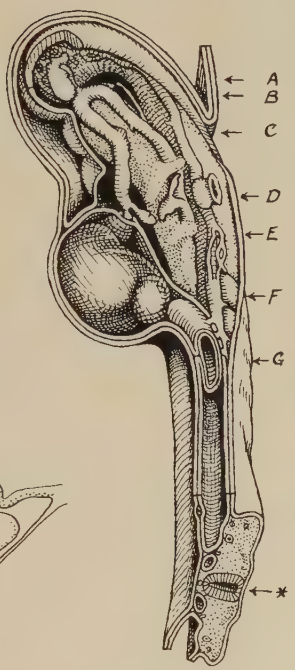
Beginning of amnion formation. It will be recalled that there has been no Rauber's layer in the opossum at any time. The cells from which the surface of the embryonic body is formed have been superficial from the beginning. Also the vesicle has not formed any association with the endometrium, but floats freely in the 'uterine milk.' Amnion formation, therefore, is not complicated by the presence of any extraneous material, or by any implantation process.

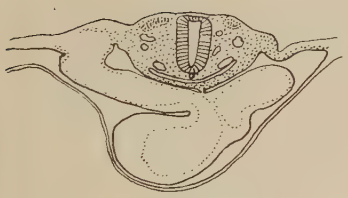
The homology of the unilaminar and bilaminar blastocysts with the blastula and gastrula of the lower vertebrates has already been commented upon. The entire external lining of the vesicle is ectodermal. The so-called trophoblast is merely the primitive ventral ectoderm. The internal lining of the vesicle is entirely endodermal, at first corresponding to the archenteron, and later to the enteron plus the yolk sac. When mesoderm forms at the animal pole, it separates ectoderm and endoderm. As it spreads, it extends this separation throughout one-third to one-half of the vesicle; but it never invades the ventral half. Along an elongate, elliptical line enclosing the animal pole the mesoderm splits tangentially to form the somatopleuric and splanchnopleuric layers with the coelomic space between them. Part of each of these layers is to be incorporated into the embryonic body and part to be discarded, but there is no visible distinction between the embryonic and accessory cells. Finally, the most dorsal mesoderm, which is within this ellipse, is again unsplit.

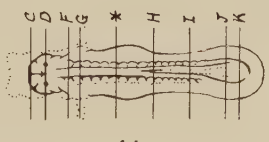
At stage 26, therefore, the original bilaminar vesicle remains unmodified in its ventral half to two-thirds. The next most dorsal portion including the layer of unsplit mesoderm is the area vasculosa or vascular portion of the yolk sac. The definitive embryonic body is developing approximately at the center of this vascular area, and the coelom is confined to a narrow elliptical strip in the immediate vicinity of the embryo.



Stage 26
Late Ninth Day







E.M.S.C., Jr.

Fig. 28 Lateral view of reconstruction of stage 26 (16146) and sections from 16145.

In the early part of the ninth day (stage 24) the embryonic head begins to grow forward beyond the coelomic ellipse so that it comes to overlie a portion of the area vasculosa, and simultaneously the mesoderm in this region (i.e., the floor of the subcephalic pocket) becomes resorbed. The portion of the yolk sac which is thus inside the area vasculosa but devoid of mesoderm (the proamnion) is the portion into which the head dips when the cephalic flexure forms. The layer of ectoderm and the layer of endoderm which thus come to enclose the head constitute the head fold of the amnion. Laterally and posteriorly the embryo is still surrounded by the coelomic ellipse, but anteriorly it reaches over the coelomic region and dives head first into a pocket in the yolk sac.

In the opossum, and generally in the Sauropsida, the cephalic flexure seems to play a mechanical part in making the head fold form before the tail fold, but this is possible only where amniogenesis begins at a late stage of development. In mammals the general tendency is for the amnion to form at an early stage, and 1) to dispense with folds altogether (as in man where it forms at a stage corresponding approximately to the opossum stage 14); or 2) to form from folds simultaneously on all sides (as in the pig where the time corresponds approximately to the opossum stage 21 or 22); or 3) to be slightly more advanced at the caudal fold (as in the rabbit where the time is very nearly the same as in the opossum). The opossum thus conforms to the reptilian or avian type in time of amnion formation and in precocity of the head fold, but probably these relationships are not very significant.

Selenka (loc. cit.) was the first to point out the unique constitution of the head fold of the amnion in the opossum. The fact that the proamnion, which is very transitory in other animals, is persistent in the opossum leads to a very complicated situation when all the amniotic folds meet during stage 29.

Fusion of the hearts in the ventricular region. In stage 25 the concrescence of the lateral body walls in the head region

resulted in the fusion of the heart tubes in their bulbar portions, and the formation of a pharyngeal floor as far caudad as the second branchial pouch. During stage 26 this same process carries the fusion of the hearts through the ventricular region, and the floor of the pharynx beneath the posterior branchial complex—a dorsolateral outpocketing of the pharynx from which the third, fourth and fifth branchial pouches will be derived (fig. 28, reconstruction). At the same time the heart twists slightly so that the bulb is pushed to the right and the ventricle to the left. The atria and sinuses of the hearts are still separate, and the floorless portion of the pharynx immediately between them is the portion in which the lung pouches are forming.

Origin of the postcardinal vein. The general manner of origin of the postcardinal has already been indicated in a previous section. It is part of a primary longitudinal venous complex which originates beneath the floor of the mesencephalon and grows caudad on the medial side of the sensory ganglia and the otocyst. As it reaches the level of the anterior end of the umbilical vein it forms capillary communications with the latter and thereby gains access to the sinus venosus; but other capillary sprouts from it continue caudad to the anterior level of the limb ridge—fourth somite. Here they invade the dorsal part of the limb. This seems to be their primary association in this part of the body, but as the nephric anlagen are developing from the seventh somite caudad, the postcardinal venules, still growing caudad, soon become associated with them also. In later stages the postcardinal retains its connection with the dorsal part of the forelimb and establishes a similar connection with the dorsal part of the hindlimb, but the association with the developing mesonephros becomes much the most important.

There is still one group of branches from the postcardinal plexus which have not been mentioned. At intersomitic levels capillary sprouts run dorsomedially between the somites and communicate with similar sprouts from the aortae. These are the precursors of the segmental veins.

Miscellaneous details. The first aortic arch is no longer plexiform, and its branches near the optic lobe have become organized into the ophthalmic artery. Between the first and second gill pouches plexiform branches from the ventral and dorsal aortae are uniting to form the second aortic arch (fig. 28, reconstruction and section D). Posterior to the level of the eleventh somite many small vitelline branches leave the aortae and pass into the area vasculosa (sections I, J, K). From this plexus the vitelline or superior mesenteric artery will later be derived. There are segmental branches from the aortae at all intersomitic levels.

The area vasculosa is rich in blood islands and includes a continuous plexus of small vessels connecting the sinus terminalis with both the vitelline veins and the vitelline arteries, so that dislodgement of the blood cells from the islands and continuous circulation will begin as soon as the heart starts beating.

The forelimb plate has thickened into a ridge which overhangs the more lateral somatopleure.

Comparative notes. The 9-day-old rabbit (e.g., Minot's specimens 619, 568, 570) is more advanced than the opossum of the same age in having its brain roofed over, and in having a caudal amniotic fold. It is less advanced in number of somites, in the stage of development of the optic and otic anlagen and of the pharyngeal pouches. In other respects it is very similar.

A 1.77-mm. lizard (Lacerta agilis—Peter's specimen no. 42) is slightly ahead of the stage 26 opossum in the development of the head amnion, and in having a rudiment of the allantois. It is very decidedly behind the opossum in somite number, in the development of the eye and ear, in the pharyngeal pouches, and most of all, in the vascular and urinogenital systems, which can hardly be said to have begun in the lizard.

A 33-hour chick (10 or 12 somites—Keibel's no. 19) is similar in the development of the optic anlagen, and in the vascular and urinogenital systems. It is more advanced in

the roofing of the brain, and it is less advanced in the differentiation of the head amnion, the pharynx, the somites and the otic anlagen.

General comments on comparative data. In the comparative notes I have tried to select a representative type for each of the principal groups of amniotes except the montremes, for which last-mentioned the available data are insufficient. The lizard was chosen to represent the cold-blooded Sauropsida because it is considered the least specialized living reptile (Parker and Haswell, vol. 2, p. 338). The chick was chosen to represent the warm-blooded Sauropsida because it is the best known bird. The rabbit was selected for the placental mammal, not only because it is fairly unspecialized, but also because for the first 10½ days of gestation it has almost precisely the same developmental rate as the opossum.

The existence of the term Sauropsida implies that the reptiles and birds have more in common with each other than either has with the mammals. In general this is probably true, but some conspicuous exceptions were listed in the comparative notes in this chapter. The vascular and urinogenital systems appear in the lizard at a much later stage of development than they do in either the marsupial or placental mammal. In this case the other sauropsidan, the bird, accords with the mammals, not with the lizard. The obvious implication of these facts is that the early differentiation of these two systems is not a mammalian in contradistinction to a sauropsidan feature, but is an homoiothermal in contradistinction to a poikilothermal characteristic. Actually neither the bird nor the opossum can control its body temperature at birth, but both of them are 'incubated' until they can. This parental attention enables them to maintain a constant temperature and a high rate of metabolism. It seems very likely that the high rate of metabolism in the warm blooded animals makes necessary the early development of the circulatory and excretory mechanisms.

There are some features in which the opossum is different from all the other forms with which I have compared him.

For instance, the pharynx develops gill pouches and lung rudiments at an earlier stage than in any of these other animals. That the lung begins its development early is not surprising, for the lung must be put to use in the opossum at an extremely early stage of general body development. The chick begins to breathe during the eighteenth day of incubation, which corresponds to about the twenty-fourth day after mating in the opossum; the rabbit begins to breathe during the thirtieth day; but the opossum has been breathing since the thirteenth day! The opossum at this time corresponds in general development to a chick of about the seventh day of incubation, or a rabbit of about the fifteenth day of gestation.

The reason for the precocity of the branchial pouches is perhaps less obvious; but, if the theory that the lungs are really modified gill pouches be correct, then part of the explanation may be that the whole branchial apparatus begins development early because the lungs, which are part of it, must be used early. But, regardless of the origin of the lungs, much of the essential mechanism of sucking and breathing, e.g., the larynx, is derived from the branchial arches, which must, accordingly, be precocious.

Other unique features in the opossum are the precocious development of the myelencephalon, the cervical cord, the anterior somites (the opossum has about 13 somites before the neural folds make contact anywhere), and the ear primordium. These features, I believe, are all associated with the early birth of the marsupial. The myelencephalon is precocious because the nerves to the sucking mechanism (the tongue, etc.), the lungs, and the stomach, must be used at an early stage. The cervical cord must be rushed ahead to assist in the sucking mechanism, to provide a brachial plexus to control the forelimb (by means of which the opossum gets into the pouch), and to provide a phrenic nerve to move the diaphragm and make breathing possible. The anterior somites must be developed at a rapid rate to provide musculature for the forelimb.

But what about the early development of the ear? The opossum has no functional vestibular apparatus until about 41 days after birth, and cannot hear until about the fiftieth day. So there would seem to be no need for an early start in the differentiation of the otocyst.

I believe the explanation lies in the fact shown by a number of operative experiments on amphibians—that the otocyst is induced by the underlying tissues. The hyoid mesoderm, according to Harrison ('35), is the primary inductor, and the hind-brain a secondary inductor. If the inductor is precocious, the induced organ will be precocious. We have already seen that both of these inductors are precocious for other reasons. Paradoxical as it may sound, the ear develops early, not because the opossum will have to hear at an early stage, but because he will have to breathe, and suck, and use his stomach at an early stage.

X. THE TENTH DAY. Stages 27 to 29

The first third of the tenth day

Stage 27. External distinctive features. The cervical flexure. The lung buds. The tail fold of the amnion. Changes in the vascular system. Miscellaneous details.

External distinctive features. Stage 27 is distinguished externally from stage 26 by the cervical flexure and the tail fold of the amnion; from stage 28 by the fact that the anterior neuropore and the otocysts are still wide open.

Associated with the cephalic and cervical flexures are changes in the extent of the head fold of the amnion. In stage 26 the cephalic flexure had occurred in the region of the midbrain and the head had become enclosed in the pro-amnion back to the level of the otocysts. In stage 27 the cervical flexure occurs at the level of the cervical cord, the heart, and the first somites, and the head fold passes over the otocysts and back to this new level. The caudal fold, which will be described below, first appears in stage 27 and covers part or all of the sinus rhomboidalis.

The cervical flexure. The flexures which play such a conspicuous part in changing the external as well as the internal appearance of the embryo between stages 26 and 35 seem to be primarily associated with local spurts of growth in the central nervous system. The first flexure is the cephalic, which was described in connection with stage 26. This seems to be due to a lengthening of the mesencephalic plate which causes it to buckle and lift dorsally at the level at which the

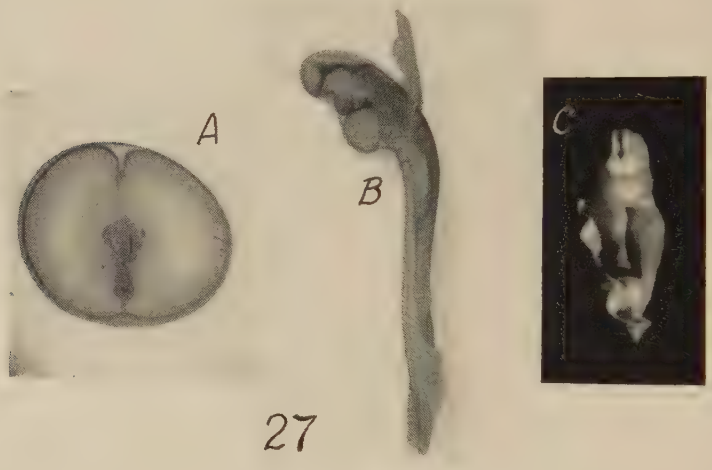


Fig. 29 Photographs and drawing of stage 27. A, ventral view of 16148 after removal of non-vascular chorion showing posterior intestinal portal, and open anterior neuropore. B, drawing of specimen 17141, showing caudal amniotic fold and open otocyst. C, photograph of caudo-ventral view of 17185 showing open anterior neuropore and anterior intestinal portal.

nuclei of the third and fourth cranial nerves and the nucleus ruber and the substantia nigra will later appear.

During stage 27 two new growth centers appear in, 1) the alar plates of the mesencephalon, and 2) the cervical cord. The growth of the alar plates (from which the corpora quadrigemina develop) causes the mesencephalic walls to rise dorsally and close into a tube before either the prosencephalon or the metencephalon have closed over. The growth of the cervical cord causes the central nervous system to bend and

lift away from the pharynx at the level of the lung buds (fig. 30, reconstruction and sections B and C). This is the cervical flexure. Both it and the cephalic flexure are conspicuously reflected in the external body form.

The lung buds. The earliest anlagen of the lungs were mentioned in chapter IX where they were pointed out in stages 24 and 25 as first, the mesothelial thickenings, and then, associated local endodermal outpocketings from paired longitudinal branchial furrows. In figure 31 these furrows can be followed to the point where the lung pouches are located at the dorso-lateral corners of the pharynx, which is just in process of acquiring a floor at their level (stage 27). Attention has already been called to the striking similarity between the lung pouches of stage 25 and the second branchial pouches of stage 24+.

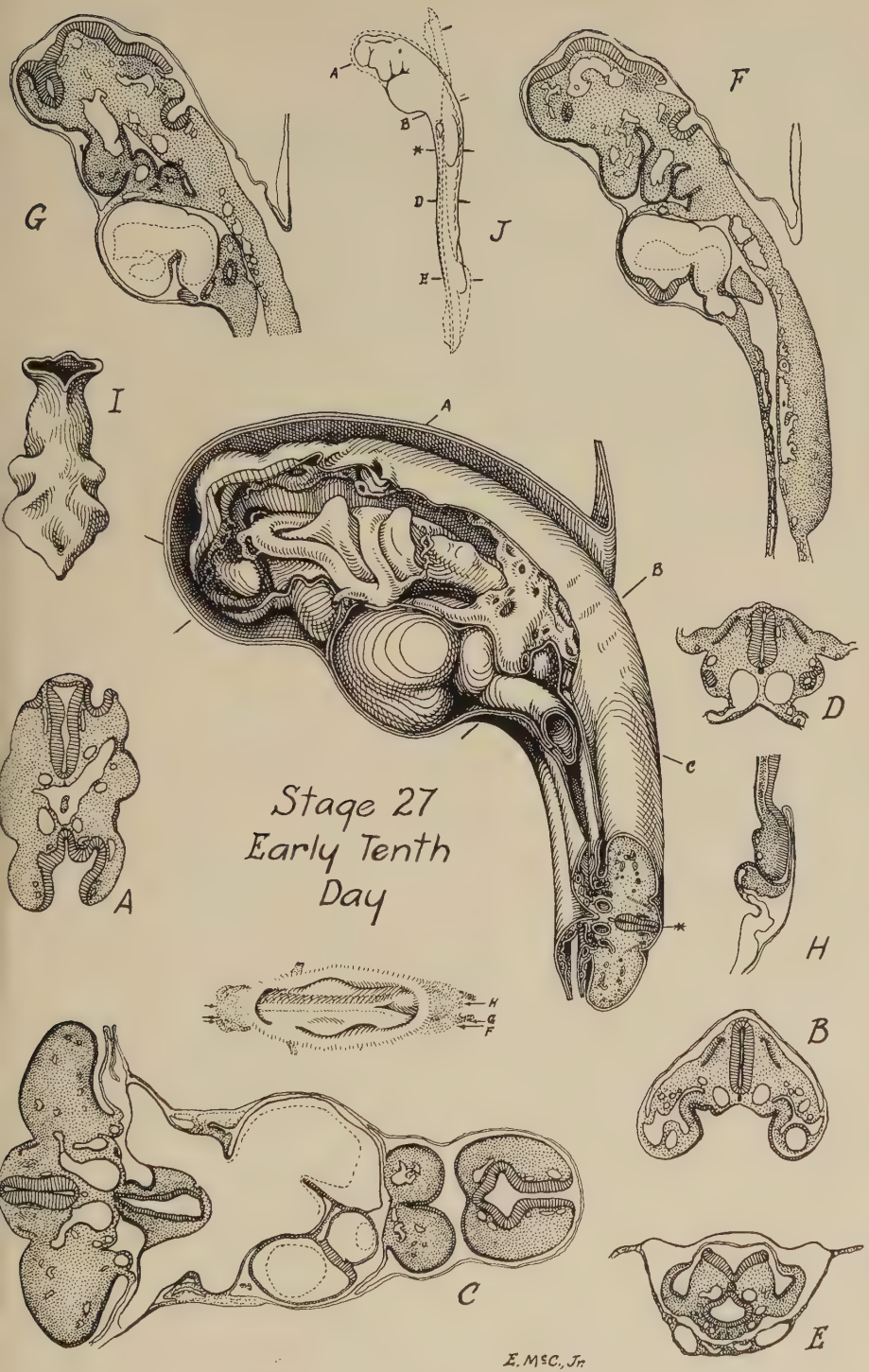
Figure 32 shows photographs of models of the pharynx taken from early and late specimens of stage 27. The early specimen (A) shows a pharynx which is triangular in section at the lung level, with the apex placed ventrally between the venous sinuses, and the lungs situated at the dorso-lateral corners of the triangle. The roof of the pharynx is beginning to lift into a dorsal ridge which forms when the central nervous system and the notochord lift dorsally as just described in connection with the cervical flexure. The late specimen (B) shows the ventral apex obliterated (by the concrescence of the atria of the hearts) and the dorsal ridge lifted so high that the lung buds are now at the ventro-lateral corners of a new triangle.

These changes are represented from the lateral view in figure 33. The branchial ridge illustrated here is, of course, the outside view of the branchial furrow previously referred to. The rise in the floor of the pharynx beneath the first branchial pouches in stage 25 is due to the fusion of the anterior end of the heart tubes into a single median bulbus cordis. The ridge labeled dorso-mesocardial line indicates the position of the mesocardium of the atrium, which does not meet its fellow of the opposite side in the midline until the end of stage 27.

The branchial pouches arise as successive local outpocketings of the branchial ridge from the anterior end caudad. In the earliest specimen here shown (stage 25) the first pouch is well developed and the second is distinctly recognizable. In stage 26 in addition to these two a new outpocketing is seen, which is labeled posterior branchial complex, and which represents the fused anlagen of the third, fourth, and fifth gill pouches. As the third is separating from this complex in stage 27, a still more caudal pouch (l.p.) in this same ridge forms the anlage of the left lung. The line across it in the figure shows the location of the cut end of the model seen in figure 32, A.

At this stage the atria of the heart tubes are still separate and the deep ventral groove in the pharynx lies between them. On first sight this groove appears to be the laryngo-tracheal rudiment, but an examination of the late specimens of stage 27 shows this not to be the case. For when the atria come together in the midline (fig. 32, B, and fig. 33, stage 27+) this groove is completely eliminated and the floor of the pharynx is pushed up to the level of the lung pouches. Meanwhile, the roof of the pharynx, which was depressed by the central nervous system in stage 25 (see also fig. 31, second branchial pouches), has lifted dorsally as the central nervous system moved away from it in connection with the cervical flexure, so that now the lungs are at the ventro-lateral corners of a new triangle. In stage 28 the lung buds and the branchial ridges begin to pinch off from the more dorsal part of the pharynx from behind forward, so that in stage 29 a distinct trachea is formed from the fusion of the posterior portions of the original lateral branchial ridges. This is in keeping with the fact that the cartilages of the trachea, which will form in stage 34, are generally regarded to be of branchial origin.

Fig. 30 Reconstructions and sections of stage 27. The central reconstruction is from 17141 as are also sections G, F and H. Sections A and B are from 17142, and sections C, D and E and reconstruction I are from 17154.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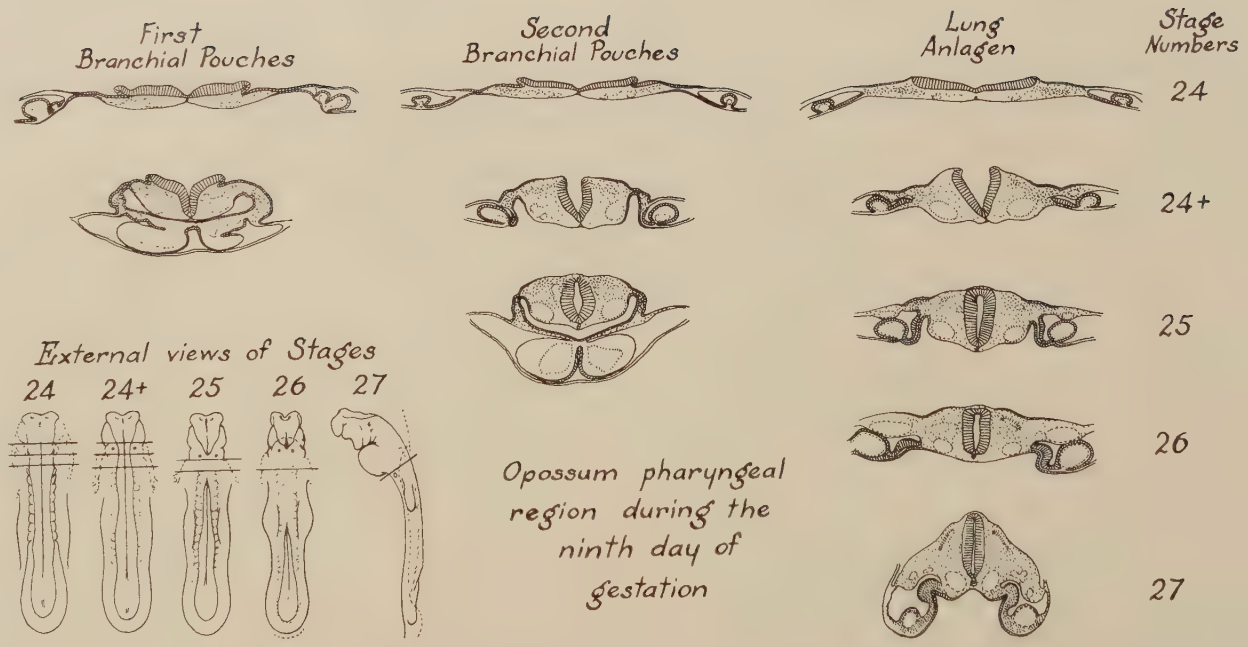


Fig. 31 Sections to show early stages of branchial pouches and lungs.

Fig. 32 Photographs of wax models of pharynx of early and late specimens of stage 27. A, early specimen (17154) showing lung pouches at dorsolateral corners of pharynx. B, late specimen (17142) showing lung pouches at ventrolateral corners of pharynx.

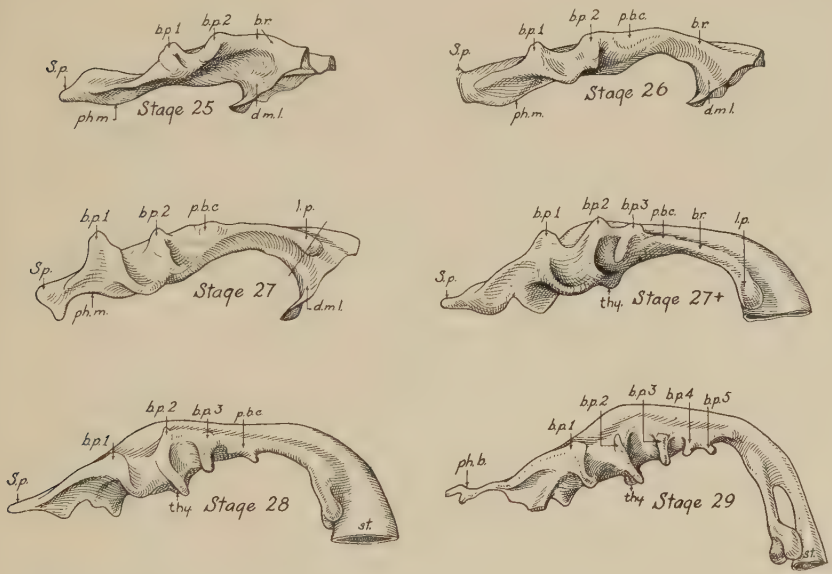


Fig. 33 Lateral reconstructions of the pharynx from stage 25 through stage 29 to show the development of the branchial pouches and lungs.

As in all other mammals, the pulmonary arteries arise (see stage 30 in the opossum) as outgrowths of the sixth aortic arches, or if you interpret it that way, as posterior extensions of the ventral aortae—at any rate, as *branchial* arteries. The nerves to the lungs, the vagi, are universally considered to represent a fusion of the posterior branchial nerves. All of these facts have led me to believe that the lungs are modified gill pouches.

According to Goodrich ('30) this theory dates back to Goette (1875). After a considerable period of neglect it has been revived and championed by Makuschok ('11, '12, '13) and others. Evidence (not always recognized as such) has accumulated from Amphibia, Reptilia, Aves, and Mammalia, i.e., all the pulmonate groups; but two objections have been urged. First and most important is Greil's opinion that the lung rudiments, though often paired and lateral in all these groups, are in all cases that he knows too ventral to be homologous with gill pouches. That objection seems best answered by the evidence from the opossum just presented, in which the lungs at the beginning are definitely dorso-lateral, not ventro-lateral, and are part of a conspicuous branchial ridge. The second objection is simply that the lung pouches never make contact with the ectoderm. This latter does not seem to be an important criterion for there are other cases in which gill pouches fail to make contact with the ectoderm, particularly the more posterior ones—for instance, the sixth in Triton (Makuschok, '11) and the fourth and fifth in the rat (Pischinger, '32).

Having seen, therefore, that the lungs themselves arise in the same manner and from the same source as the gill pouches, that their blood supply and nerve supply are branchial, that the cartilages of the trachea and bronchi as well as those of the larynx are of branchial origin, and that in the face of all this confirmatory evidence no important contradictory evidence has been found, there seems to be no escape from the conclusion that the lungs are modified gill pouches. Makuschok has shown the same to be true of the

swim bladder of fishes; so the long accepted theory of the homology of the lungs and the swim bladder is not contradicted by this interpretation—they are homologous, and they are both gill pouches.

The tail fold of the amnion. The anterior and the posterior amniotic folds in the opossum are of different constitution and never completely merge. The definitive amnion thus has two quite distinct portions, which Selenka (loc. cit.) recognized and accurately described.

After pointing out that the pro-amnion, which van Beneden and Julin first described in the rabbit and the bat, is a transitory structure which contributes nothing to the definitive amnion in all other known cases, he says:

Beim Opossum umhüllt das aus Ekto- und Entoderm bestehende Kopfamnion (wie ich das von Van Beneden und Julin als 'Proamnion' beziechnete Gebilde nennen will) vier Tage nach Beginn der Furchung [stage 27] ungefähr des vordere Drittel des Embryonalkörpers, wahrend das Rumpfamnion, welches aus Ekto- und Mesoderm zusammengesetzt ist, dessen hintere zwei Drittel umfasst. Am Ende des fünften Tages [stage 29] sind beide Falten fast gleich gross und am Ende des sechsten Tages [stage 32] ist der ganze Embryo ausschliesslich vom Kopfamnion (Ekto- und Entoderm) umkleidet, während das Rumpfamnion sich hinter den Schwanz zurückgezogen hat. Das Kopfamnion spielt also hier die Rolle eines Dauerorgans, hingegen ist das Rumpfamnion das transitorische Gebilde geworden.

The closure of the amniopore is illustrated in figure 34. The germ layer constitution of the amnion can be seen in the reconstruction of stage 29 (fig. 39). The final proportions of the head-amnion and trunk-amnion referred to by Selenka are shown in stage 33 (fig. 52).

Abnormalities are fairly common among opossum embryos. I have one specimen of stage 29 which has a head fold back to the limb buds as in stage 28, figure 34, but no tail fold at all. Another specimen has a tail fold like that of a normal stage 27, but no head fold at all. That these specimens represent real abnormalities, not merely extremes of normal variation, is

abundantly clear from other anatomical features. I am fairly confident that they would never have been born, but would have been completely resorbed during the next day or so of gestation.

Changes in the vascular system. The atria of the heart tubes meet and fuse during stage 27 and the heart begins to beat. From this time on corpuscles may be found in all the blood vessels of the body. For the sake of clarity, however, they have been omitted from the section tracings.

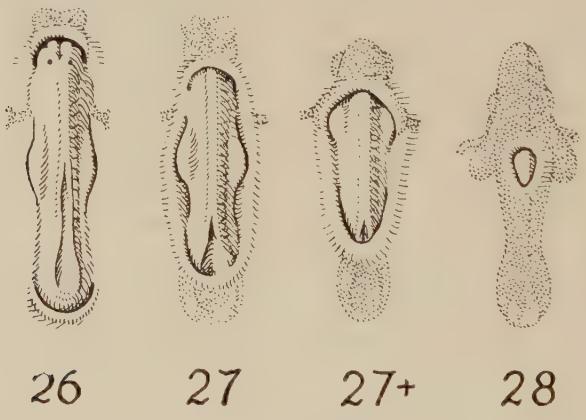


Fig. 34 Dorsal views of embryos showing closure of the amniopore.

In connection with the beginning of circulation of the blood it is interesting to note how far the vascular system has already developed. In early specimens of stage 24, as already described, there is on each side of the body a capillary plexus leading from the anterior end of the heart tube through the mandibular mesenchyme, then caudad to the end of the body and out into the area vasculosa. In later specimens of the same stage there has been a selection and enlargement of two main arterial vessels from this plexus, the dorsal aortae, and an elimination of most of the other original channels. By a similar process in stage 25 the mandibular capillary plexus is converted into two well-formed aortic arches. In stage 26 the capillary plexus in the hyoid mesenchyme shows a reduction in

the number of channels, and by stage 27 there are only the two well-organized second aortic arches at this level. The vitelline veins, the primary head vein, and the umbilical veins as far caudad as the anterior limb ridge, have also passed through similar capillary stages and by stage 27 have likewise become organized into single large channels. The point to be emphasized here is that all this has occurred before there has been any circulation of the blood.

The theory that all blood vessels are differentiated from primitive capillary plexuses seems first to have been proposed by Aeby in his 'Der Bau des menschlichen Körpers' (1868). In 1893 Thoma (Untersuchungen über die Histogenes and Histomechanik des Gefässsystems) suggested that the enlargement of certain channels and the neglect and gradual elimination of others were due to the circulation of the blood—the hydrodynamic theory. When Evans ('09 b, from which the above two references are cited) established the soundness of the morphological facts, he did not concern himself with the question of causation. Thoma's theory of the mechanism is still the only one which has been proposed, and is generally accepted today. Arey, for instance ('30), says, "The selection of appropriate channels from the diffuse capillary bed results both from the action of heritable patterns and the hydrodynamic factors incident to the blood flow" and " . . . . those capillaries from which the blood flow has been diverted, atrophy." I quote this passage because it should be emphasized that the mention of heritable patterns in no way extends or supplements Thoma's theory. Heredity, which determines all possible responses of the cells concerned, forms the basis of the process no matter what mechanical factors play a part in eliciting or provoking the special response which actually does occur. As heredity, then, in a general sense, is the basis of any and all possible explanations of the process, the only specific factor which has been suggested is the blood flow.

The experimental work of Oppel and Roux ('10) seems to have shown quite definitely that after the blood begins to

circulate, it can and does influence the size, course, and muscularity of vessels; but all of the development which occurs during the prefunctional period (Huxley's period of 'chemodifferentiation') remains totally unaccounted for. What I call attention to here is that, with one exception, every sort of change which occurs in the circulatory system at all occurs in the prefunctional as well as the functional stage. The one exception is the development of the muscular layer around the vessels. As this occurs only after function begins, there is no histological distinction between arteries and veins in stage 27.

The reconstruction of stage 27 (fig. 30) shows a number of new details in the vascular system which have not yet been mentioned. The capillary plexus of the third aortic arch is in process of forming. Capillary twigs from the vena capitis medialis can be seen extending laterally around the otocyst. The great venous plexus near the heart represents the proximal ends of the anterior and posterior cardinal veins, the duct of Cuvier, and the paracardinal plexus. The umbilical vein, which enters this plexus from behind and near its ventral end, has been cut off at its mouth to expose the lung bud.

More or less opposite the mouth of the umbilical a new vein can be seen coming from the ventral part of the head back across the dorsal wall of the pericardium to empty into this plexus from in front. This is the inferior jugular, which is not to be confused with the external jugular or the anterior jugular of mammals. This vein was first described by Grosser ('01) in the embryo of the bat, and later ('07) shown by him to be homologous with the inferior jugular of fishes, amphibians, and reptiles. He also found it in the cat, guinea pig, and human embryos. Lillie ('08) described it in the chick as the external jugular; and Lewis ('09) described it in the human embryo as the linguofacial. It is very well developed in the opossum and remains an independent vessel until stage 32, though meanwhile its mouth migrates up the duct of Cuvier to the internal jugular vein. At this time it also acquires connection with branches from the external jugular, and its flow is soon diverted into the latter channel

so that it loses its other connections and becomes a tributary of the external jugular.

The capillary plexus which originally connected the aortae with the sinus terminalis has in stage 27 become organized into two vitelline arteries which leave the aorta in the region of the midgut, run caudad beneath the extra-embryonic coelom and through the area vasculosa to the sinus. In advanced specimens the two have fused into one in their extra-embryonic portion.

Miscellaneous details. There is a recognizable hind-gut diverticulum or posterior intestinal portal in even the very early specimens of stage 27, but no allantois. Section H, figure 30, shows the posterior end of the body of a very early specimen in sagittal section. It can be seen that a deep and pointed proctodeum makes contact with an equally pointed hind-gut. The contact is, of course, the cloacal membrane.

Anterior to the hind-gut diverticulum another pocket in the endoderm can be seen. This represents the vestige of a notochordal canal. At this point there is no limiting membrane on the dorsal side of the endoderm or on the ventral side of the ectoderm, and endoderm, notochord, and Hensen's node are continuous. Up until stage 26 there had been a distinct primitive pit in Hensen's node, but in stage 27 this pit closes. Selenka mentioned not having seen a neurenteric canal, though he thought it likely that this was due to his not having the appropriate stage. No neurenteric canal is formed, but the endodermal pit which marks the site where it nearly formed remains distinctly visible through stage 28 (fig. 37).

In the earliest specimens of stage 27 (17141, 17142) the neural tube is closed anteriorly only through the myelencephalon—the metencephalon, mesencephalon, and prosencephalon being wide open (fig. 30, A). In specimen 17154 the mesencephalon is closed but the metencephalon and prosencephalon are still open (fig. 30, C). In the last six specimens the metencephalon has closed and only a small neuropore remains in the prosencephalon at the level of the optic stalks.

In stage 28 that also closes and its location at the time of closure is seen in the reconstruction (fig. 36). The posterior neuropore, however, is very wide in stage 27 (fig. 30, E), and does not close until stage 30.

The otic cups, though still open, are in late specimens somewhat constricted at the mouth.

The third gill pouch is now distinct from the posterior complex.

Comparative data reveal the same set of relations between the different organ systems as that described in connection with earlier stages. As the generalizations based upon the early stages apply equally well to the later ones, detailed comparisons with other species will be omitted until the discussion of the condition at birth (stage 35).

The middle of the tenth day

Stage 28. External distinctive features. The primary lumbar flexure. The mesonephric tubules. The liver diverticulum. The paracardinal plexus. The veins of the limb buds. The allantois. Miscellaneous details.

Fig. 35 Photographs of external views of stage 28. A, ventral view of 16149 after removal of non-vascular chorion. B, ventral view of 16154 after removal of all extra-embryonic membranes except anlage of the body stalk. C, lateral view of 16150. D, dorsal view to show amniopore of embryo shown in A. E, ventral view of 16150 before the removal of the area vasculosa.

External distinctive features. Stage 28 is distinguished externally from stage 27 by the closure of the otocysts and anterior neuropore (fig. 35, C and B), and by the fact that the

head fold of the amnion has covered the limb buds. It is distinguished from stage 29 by the presence of an amniopore (fig. 35, D), and the absence of a deep naso-oral groove (fig. 35, C).

Though no distinct naso-oral groove has formed in stage 28 the olfactory placode is well developed and noticeably depressed. The naso-lachrymal groove, which was quite conspicuous in stage 27, is becoming less so as the maxillary process fuses with the lateral part of the naso-frontal process.

The forelimb rudiment has grown out until its length is greater than the width of its base. It is now described as a limb bud instead of a limb ridge. En route to the area vasculosa the omphalomesenteric vein sweeps around the anterior or radial border of the limb bud.

Though not confined to stage 28, perhaps the most conspicuous external feature which has not heretofore been described is the primary lumbar flexure.

The primary lumbar flexure. When the embryonic body was relatively flat (early stages up to 26) its dorsal curvature conformed to that of the rest of the vesicle—that is, it was slightly convex. During stages 26 and 27 the anterior third of the body bent sharply in the ventral direction at two points (cephalic and cervical flexures) and thus thrust itself down into the yolk sac. During stage 27 the dorsal line from the tenth somite back is fairly straight, but there is often a slight concave flexure in the posterior third of the body. This is the beginning of the primary lumbar flexure.

During stage 28 this flexure becomes very marked. It extends from about the tenth to the twenty-sixth somite and is deepest at the level of the twentieth to the twenty-second. It occurs at the time of constriction of the amniopore. The amnion at this time may be compared to an open bag with a loop of string tied around the mouth, which loop is gradually being tightened so that the opening (the amniopore) is being closed and the bottom of the bag (which includes the flat thoracic and lumbar parts of the embryonic body) is being

folded into a deep concavity. When the amniopore is actually closed (during stage 29) the concave bending stops, and very shortly thereafter (stage 30) the flexure reverses itself. I mention this only as a simile, for it is difficult to imagine a force which might correspond to the closing of the string loop.

In the human embryo of the early part of the fifth week there is a similar concave curvature of the back. The amnion in that case has formed very much earlier and not by a folding process at all, which may be taken as evidence that there is no causal relation between the origin of the amnion and the formation of this flexure. To explain this case it has been suggested that the 'weight' of the yolk sac, which 'hangs' from this point, is responsible for pulling the back down. I don't know whether the yolk sac has any weight or not in the fluid in which it is immersed, also I don't know just what 'hangs' means when the site of implantation may be dorsal or ventral with equal frequency and is often lateral. But the final answer to the weight-of-the-yolk-sac theory comes from the opossum in which the yolk sac is the lining of the entire embryonic vesicle, and the embryo actually descends into and becomes completely surrounded by its own yolk sac. Nonetheless, the opossum embryo develops a good primary lumbar flexure, which obviously in this case cannot be caused by any weight of the yolk sac.

More likely than a connection with either the process of formation of the amnion or the weight of the yolk sac, is the possibility that, like the other flexures, this one is due to local differences in growth rates. If so, at the time of its formation growth in the thoracic and lumbar regions should be more rapid on the ventral than on the dorsal side of the body. There is good reason to think this is so, for the rapidly developing mesonephros is not only at exactly the right place, but in both man and the opossum it is at exactly this time developing tubules which are lengthening rapidly and being twisted into S-shapes for lack of space. This is also true of the 55-hour chick, which corresponds to a stage 28 opossum.

and which also has a well-developed primary lumbar flexure, though here the picture is somewhat complicated by the fact that the anterior part of the body, being unable to dip into the dense yolk, is forced to twist to one side. The rabbit and the lizard, which do not show the flexure, also do not have any mesonephric tubules developing at this time. In these cases the tubules form later on when there are other forces at work which compel the mesonephros to expand by projecting into the coelom instead of by flexing the back. The 'other forces' referred to will be discussed in connection with stage 30.

The mesonephric tubules. In the description of the nephrogenic ridge during late stage 24 the earliest anlagen of the mesonephric tubules were mentioned (p. 67 and fig. 22). The anlagen at that time were differentiating within a continuous longitudinal ridge. They consisted simply of a series of small, separate lumina along the center of the thickest part of the ridge. The cells around the lumina were radially disposed, but between adjacent lumina the cells showed no recognizable organization.

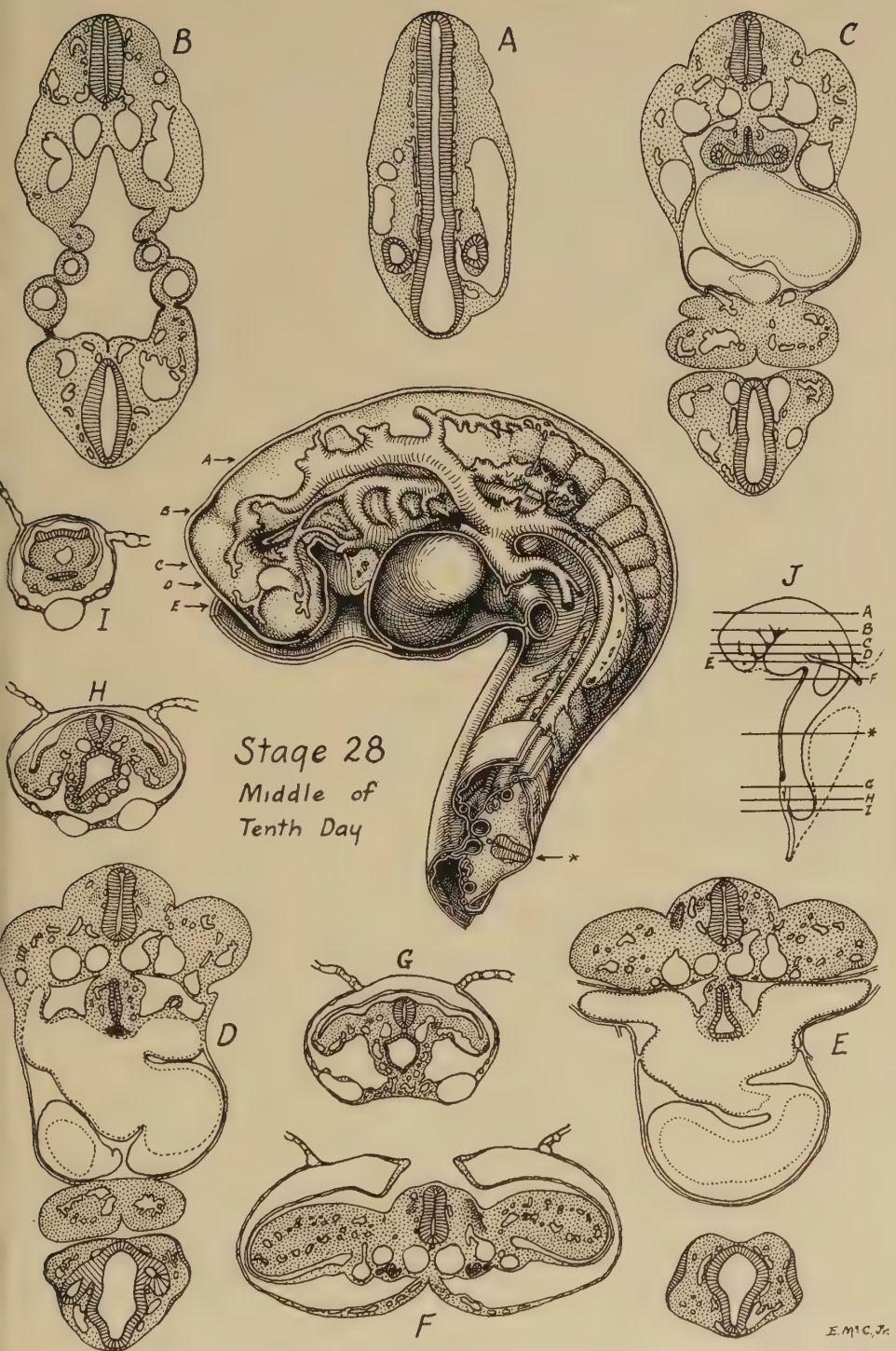
In stage 28 two important developments take place. First, the ridge pinches off from the coelomic epithelium altogether, thus becoming a nephrogenic cord. And second, the mesonephric tubule anlagen begin to separate from each other, increase rapidly in length, and become bent into double curves to accommodate themselves to the inadequate, available space. Though the adjacent tubule anlagen separate from one another (probably by the organization of the intermediate cells about the separate lumina), they do not separate from the most dorsal part of the original ridge. This once dorsal edge has now become lateral as a result of the rounding up of the body. Though it itself remains unsegmented and for the most part at stage 28 still uncanalized, it never loses continuity with the walls of the tubule anlagen which differentiate out of the same longitudinal anlage. It is, of course, the wolffian duct, which is thus continuous with all of the pronephric and mesonephric tubules from the very beginning.

The changes just described occur in cephalocaudal progression, and are correspondingly most advanced in the anterior mesonephric segments. In stage 28 the tubule anlagen are S-shaped from the tenth somite (fig. 36, section F) back to about the eighteenth. From the eighteenth to approximately the twenty-fourth the tubules are separate from each other but are not long enough to be very much twisted, and the most posterior of them are more sac-like than tubular. Caudad from this point they merge into a continuous nephrogenic cord much like that described for the region of the eleventh somite in stage 24. It was a section through a tubule anlage in this cord that Selenka (pl. XXII, fig. 13) mistook for the wolffian duct. And finally, the cord merges into the undifferentiated mesoderm beyond the last somites. At the level of the notochordal canal (W.I.C. 16149, sections 913 to 918) this undifferentiated mesoderm merges with the endoderm which has no basement membrane.

The pronephros at this time is about as well developed as it will ever be. It consists of five or six very slender, solid 'tubules' which are connected with both the coelomic epithelium and the wolffian duct. They are situated between the seventh and tenth somites (fig. 36, section E), and occur at both somitic and intersomitic levels. In specimen 16149 on the left side there are about thirty-six nephric tubules in all, of which the first six are pronephric, and the last thirty mesonephric. This count includes the most posterior anlagen which are not separate from one another except in their lumina.

The liver diverticulum. A diverticulum from the foregut in the midventral line at the level at which the omphalomesenteric veins empty into the sinus venosus, is the first anlage of the liver. It is shown in cross section in figure 36, section D. During stage 28 it is a simple diverticulum without any liver cords branching from it. It is found also in a few of the late specimens of stage 27, that is, specimens in which the otocysts and anterior neuropore are still open.

Fig. 36 Lateral reconstruction and cross sections of stage 28. The reconstruction is from 16150, the sections are from 16149.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The paracardinal plexus. Part of the originally extensive venous plexus from which the duct of Cuvier and the proximal ends of the pre- and post-cardinals differentiate persists in the angle between the cardinals until stage 32 when the jugular lymph sac begins to form in this region. This persistent portion is called the paracardinal plexus, and its fate will be discussed in detail in connection with stages 32 and 33. It is seen in the reconstruction (fig. 36) to have its principal connection with the precardinal, not very far from the cardinal anastomosis. It lies immediately beneath the dermo-myotomal plates, is constricted at the intersomitic levels, and communicates by small twigs (not shown in the figure) with the segmental veins. The first somite has disappeared, and only a trace of the second is left, but the sites of both may be identified by the segmental vessels and by the bulges in the paracardinal plexus, which is quite sinusoidal in the somitic regions at this time.

The veins of the limb buds. It was mentioned in connection with the origin of the umbilical vein in stage 24 that its first association is with the anterior limb bud, on the ventral or radial side. Also it was pointed out that the postcardinal in stage 26 established communication with the dorsal border of the limb ridge. Thus when the blood begins to circulate in stage 27 the rich venous plexus which ramifies through the limb drains dorsally into the postcardinal and ventrally into the umbilical.

At first the plexus seems altogether irregular, but by stage 28 it is beginning to show a more orderly arrangement of the vessels. The veins form a sort of subperipheral layer around the limb bud (fig. 36, D and E), the dorsal part of this layer draining by many small mouths into the postcardinal, the ventral part in the same way into the umbilical. The arterial supply from the dorsal aorta enters the medial part of the limb and communicates through capillaries with all parts of the subperipheral plexus.

Caudad of the anterior limb bud both the umbilical and the postcardinal veins extend all the way back to the end of the

body. Here on either side of the rhomboidal sinus they unite in a very rich plexus through the region in which the posterior limb ridge is forming (sections G and H).

The allantois. Section H also shows a ventral diverticulum from the hind-gut, which is the allantois. Its vascularization is very rich, as the figure shows, and is derived from the following sources. The arterial supply is from the dorsal aortae. In the region of the mid-gut the aortae give off innumerable mesenteric arterioles (shown in the reconstruction). Just before reaching the hind-gut two exceptionally

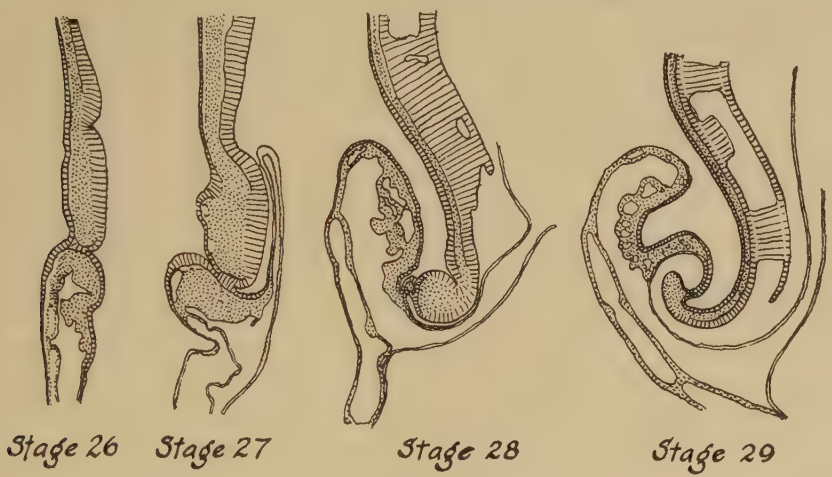


Fig. 37 Sagittal sections of posterior end of embryos from stage 26 through stage 29 to show cloacal membrane, caudal amniotic fold, extra-embryonic coelom, hindgut, allantois, and the absence of any neurenteric canal. The section for stage 26 is from 16146; for stage 27, 17141; for stage 28, 16150; and that for stage 29 is a sagittal reconstruction from the cross sections of 16154.

large branches, the vitelline arteries, descend into the walls of the yolk sac, run caudad beneath the body (G and H), and finally unite into one (I) which courses through the area vasculosa to the sinus terminalis (see also fig. 35, A and E). Posterior to these, more arterioles are given off, and those beyond the posterior intestinal portal run into the walls of the hindgut and thence into the allantois (H). The posterior wall of the allantois (prospective ventral wall) is not surrounded

by coelom, but is continuous with the ventral body wall, which is just in process of forming anterior to the cloacal membrane (fig. 37). At this point of continuity with the body wall the vessels of the allantois meet and empty into the umbilico-postcardinal plexus already described in connection with the posterior limb ridge. The developing allantois thus has a venous connection with the umbilical veins at its very inception, but not with the vitelline or omphalomesenteric veins. These latter vessels are at first almost exclusively vitelline, and what few, small, mesenteric branches there are, are too short to cross the wide mid-gut and so do not reach the allantoic region. The subintestinal vein, which forms later and acquires a connection with the allantois, will be described in connection with stage 32.

Miscellaneous details. The anterior neuropore completely closes during stage 28. The reconstruction in figure 36 shows the last vestige of it at the extreme anterior end of the prosencephalon. The posterior neuropore, however, is still in the form of a flat rhomboidal sinus (fig. 36, I). The first traces of a marginal layer outside the mantle layer in the central nervous system, indicate that axones and dendrites are beginning to grow out from the nerve cells. However, the cranial and spinal sensory ganglia are still unattached.

The vascular system shows many new features. The capillaries which surrounded the otocyst in stage 27 have now shifted the principal flow in the primary head vein to the lateral side of the otocyst (fig. 36, A). Posterior to the otocyst and anterior to the paracardinal plexus the head vein receives some rather large tributaries from the surface of the central nervous system. These are the posterior cerebral veins which play an important part in the formation of the venous sinuses of the dura mater. A similar set just anterior to the otocyst constitutes the middle cerebral group, and a third set from the mid-brain and forebrain is the anterior cerebral group. These three groups become more sharply demarcated from each other in later stages. The stub of a

small branch of the anterior cerebral shown in the reconstruction (fig. 36) running ventrally at the isthmus, is the transverse hypophyseal vein, which crosses the midline and unites the veins of the two sides.

The first aortic arch has become much more slender, but is still a single channel, not a plexus. The second aortic arch is now the largest; the third is quite large; the fourth is complete, but small; and the fifth, which in most mammals forms late or not at all, is in process of forming on time (fig. 36).

The first three gill pouches are well formed and in contact with ectoderm (fig. 36, B). The fourth and fifth are still fused (fig. 33).

The otocyst is detached from the ectoderm, and the ectoderm has thickened into a lens placode at the point of contact with the optic vesicle (fig. 36, D).

There is a slight thyroid depression in the middle of the floor of the pharynx between the second branchial pouches (fig. 33).

The last third of the tenth day

Stage 29. External distinctive features. The extra-embryonic membranes. The pharyngeal bursa. The dorsal pancreatic diverticulum. The thyroid anlage. Miscellaneous details. Comparative notes.

External distinctive features. Stage 29 is distinguished from stage 28 by the naso-oral groove, the tail bud, the club-like enlargement of the end of the anterior limb, the prominent posterior limb ridge, the closed or nearly closed amiopore with some extra-embryonic coelom anterior to its level, and the fact that the omphalomesenteric veins pass behind instead of in front of the anterior limbs. It is distinguished from stage 30 by the absence of the secondary lumbar flexure, and by the following negative facts: the anterior limb club has not flattened into a paddle; the posterior limb ridge has not become pointed enough to be called a bud; and the allantois has not enlarged enough to be noticeable externally.

The extra-embryonic membranes. Though there will be changes in the proportions of the parts in later stages, there will be no changes in the constitution of the amnion, chorion, yolk sac, and body stalk after the closure of the amniopore in stage 29.

In the description of stage 26 (chapter IX) the history of the constitution of the blastocyst wall from the earliest stages up to the beginning of amnion formation was summarized. It was pointed out that the ventral two-thirds of the vesicle is never invaded by mesoderm, and so represents a persistent

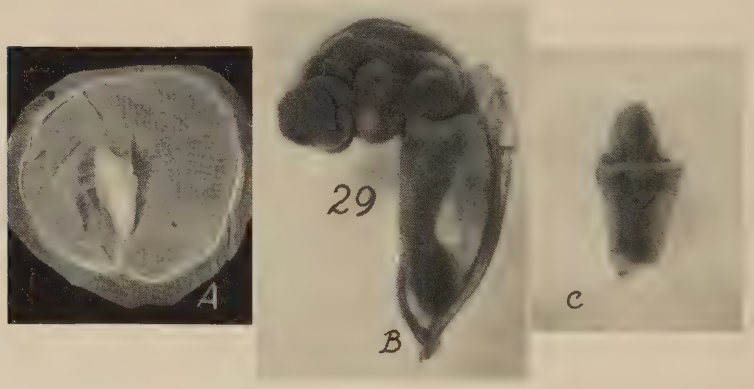


Fig. 38 Photographs of external views of stage 29. A, ventral view of 16153 after removal of non-vascular chorion. B, lateral view of 16153 with vascular chorion and head amnion also removed, but with trunk amnion intact. C, dorsal view of 16154 to show closing of amniopore.

portion of the original bilaminar vesicle—yolk sac endoderm covered by ectoderm. We shall call this the non-vascular yolk sac chorion (fig. 40, nv.ch.). In the dorsal third of the vesicle the mesoderm spreads between the ectoderm and the yolk sac endoderm, and provides a vascular supply for part of the yolk sac. This part is the area vasculosa or vascular yolk sac chorion (v.ch.), and the embryo proper is located approximately in the center of it, though slightly nearer its caudal edge. Finally, in a very restricted region within the embryonic body and immediately around its borders, the

mesoderm splits to form somatic and splanchnic layers with all the blood vessels outside the body in the lower of the two—the splanchnic layer (fig. 39, reconstruction). This leaves the surface of the vesicle non-vascular in the immediate vicinity of the embryo. I have already described how the mesoderm becomes resorbed in the proamniotic region, and how the head elongates, reaches over the coelomic loop and dips ventrally into the proamnion, thus forming the head fold of the amnion of only ectoderm and endoderm. When the caudal and lateral folds arise (stage 27) they are formed from the roof of the extra-embryonic coelom. Accordingly when they meet above the embryo and fuse, they form a non-vascular somatopleuric spot in the midst of the area vasculosa. This sheet of somatopleure underlain by extra-embryonic coelom, though of small area, is a permanent part of the outside wall of the vesicle and is of different constitution from the other two parts already described. This is the serosal chorion (fig. 40, s.ch., and fig. 39, reconstruction).

There are thus three parts of the definitive chorion in the opossum: 1) the non-vascular yolk sac chorion—ectoderm and endoderm; 2) the vascular yolk sac chorion—ectoderm, mesoderm, and endoderm; 3) the serosal chorion—ectoderm and somatic mesoderm. This last is the spot at which an allantoic placenta (such as is characteristic of all Eutheria) could, theoretically, form, if the allantois, which projects into the extra-embryonic coelom, should grow out caudally far enough to reach and fuse with the outside wall of the vesicle. But this does not occur in the opossum (see stage 33).

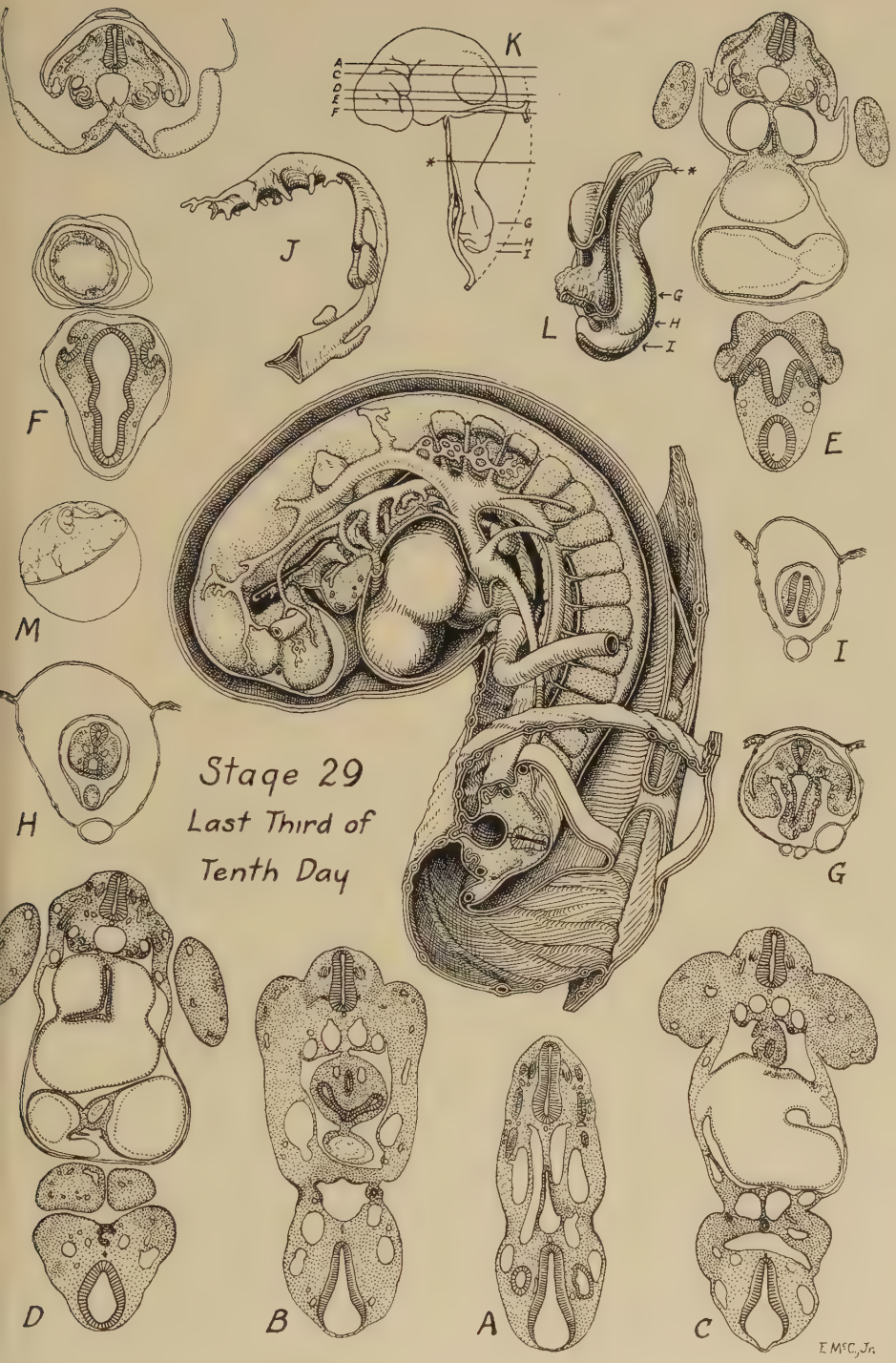
The principal peculiarities which give rise to this type of chorion are 1) the mesoderm does not spread more than one-third of the way around the vesicle; 2) the process of splitting into somatic and splanchnic layers with a coelom between them occurs in an exceedingly restricted region around the embryo proper; 3) as a consequence of the second, the yolk sac remains a permanent constituent of the outside wall of the vesicle throughout most of its area; and 4) the

allantois, instead of extending out through the extra-embryonic coelom to fuse with the serosal chorion, remains in a pocket of the yolk sac.

Though this situation is remarkably different from that found in most placental mammals, there is at least one of the latter which is sufficiently like the opossum to be a useful subject for comparison. The extra-embryonic membranes of the 10-day rabbit (fig. 40, C) differ from those of the 10-day opossum (A) in only two important points: 1) the allantois is much further developed, and is fused with the serosal chorion; and 2) the extra-embryonic coelom extends all the way to the sinus terminalis, so that the yolk sac circulation is completely separated from the surface of the vesicle. With these exceptions the opossum and rabbit are strikingly similar —especially so in having the ventral two-thirds of the vesicle composed only of yolk sac endoderm and ectoderm, and the anterior third of the amnion composed only of proamnion.

In later development the two species diverge considerably. In the rabbit (D) the mesoderm invades the proamnion and splits, so that the yolk sac comes to be completely separated from the amnion by extra-embryonic coelom. At the same time the allantois extends its fusion with the chorion throughout the serosal region, so that the allantoic vessels very literally replace the vitelline vessels. In direct contrast to this, in the opossum it is the yolk sac which enlarges and displaces the extra-embryonic coelom, so that almost the entire amnion comes to be composed of proamnion, and the vitelline vessels remain permanently in the chorion. In this situation the allantois comes to be surrounded by the yolk sac, is prevented from reaching the chorion, and serves only as an urinary reservoir (see stage 33).

Fig. 39 Reconstructions and sections of stage 29. The central reconstruction taken from 17162 shows, in addition to the embryo proper, the constitution of the amnion and area vasculosa. The sections are from 16154. The reconstruction L shows, on a slightly smaller scale, the portion of the body cut off in the central reconstruction. J shows the pharynx, lung buds, liver diverticulum, and dorsal pancreatic diverticulum. M shows the actual size of the vesicle.



Stage 29
Last Third of
Tenth Day

The description thus far has been written with primary reference to the chorion, but it has necessarily included a sort of implicit description of the amnion. It has already been

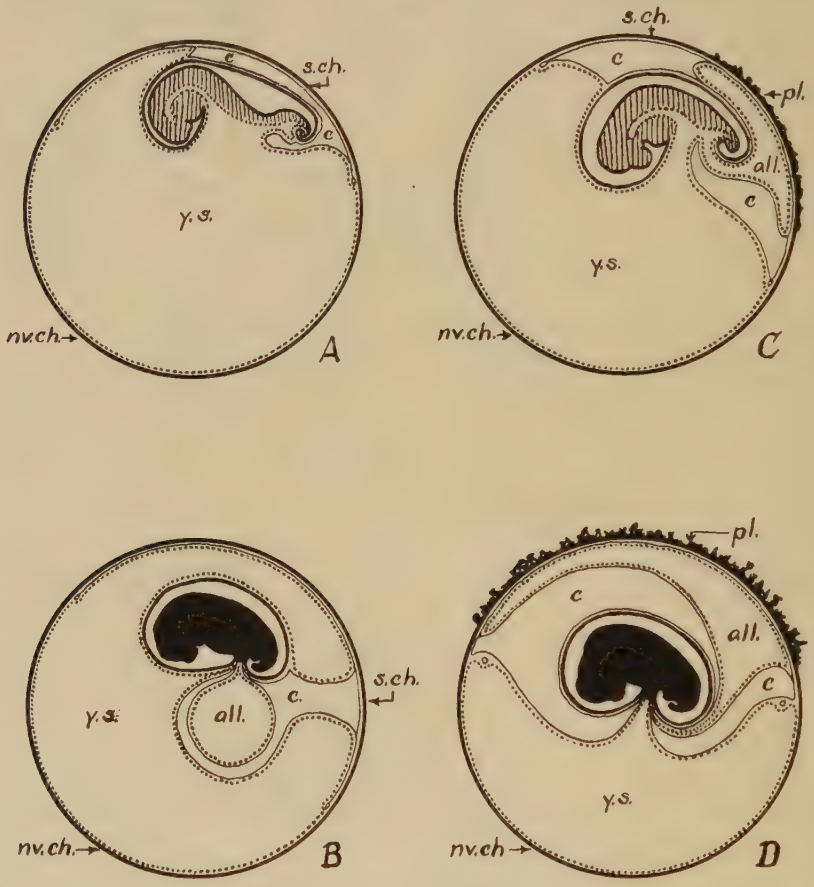


Fig. 40 Diagrammatic sections of vesicles to show the relations of extra-embryonic membranes in the opossum and the rabbit. The heavy line represents ectoderm; the light line mesoderm; the dotted line endoderm. A, opossum stage 29. B, opossum stage 33. C, rabbit at 10 days. D, rabbit at 13 days.

indicated, for instance, that the head fold is composed entirely of proamnion, and that the tail fold and lateral folds are formed of the somatopleuric, non-vascular roof of the extra-embryonic coelom.

Before the four folds meet, mesoderm invades the proamnion for a short distance ahead of the amniopore, so that the latter comes to be surrounded by extra-embryonic coelom. When the amniopore then closes (at the site indicated in the reconstruction, fig. 39) the partition or stalk which tends to form is quickly resorbed and amnion and chorion become separated from each other by extra-embryonic coelom throughout the region beneath the serosal chorion. However, as the mesoderm has invaded the proamnion for only a short distance anterior to the amniopore, its anterior extremity is marked by a two-layered membrane still connecting the amnion with the chorion. This membrane is composed of somatic mesoderm and yolk sac endoderm. If it were to break down, coelom and yolk sac would open directly into each other; but this never occurs. If traced laterally along this membrane, the somatic mesoderm will be found to meet the splanchnic mesoderm along a line marked conspicuously by the large omphalomesenteric vein coming in from the area vasculosa. As this vein always marks their line of junction it will be an interesting landmark by which to follow the later changes in proportion between the yolk sac and the extra-embryonic coelom and the corresponding parts of the amnion. Tracing the splanchnopleure, which forms the lateral wall of the extra-embryonic coelom, caudally from the omphalomesenteric vein, we find that it meets its fellow of the opposite side in the midline behind the tail bud; and that their line of junction is marked by the large vitelline artery which follows it out to the area vasculosa (fig. 39, H, I, K). There are thus three large vessels running between the embryonic body and the chorion and marking the corners of a roughly prismatic extra-embryonic coelom. The membranes which connect these three vessels to each other also connect the embryonic body to the chorion, and form a sort of prismatic, hollow, body stalk. These membranes are two-layered. The outside layer on all sides is yolk sac endoderm. The inside of the anterior face (i.e., the one between the two veins) is somatic mesoderm. The inside of the other two faces is

splanchnic mesoderm. The end of the hollow stalk is capped by a sheet of somatopleure—the serosal chorion. In stage 29 the two splanchnopleuric faces carry many small vitelline vessels (mostly arterioles from the dorsal aortae), but in later stages only the three large vessels survive.

Though I have written this description of the foetal membranes from my own observations, most of the facts had been worked out before, and accordingly a few words about the literature are necessary at this point.

Osborn's three papers on the foetal membranes of the opossum (1883 a, 1883 b, 1887) are so full of errors that it seems impossible to understand what sort of observations they could have been based upon. Even the third paper, which purported to correct the errors recognized in the first two, introduced an almost equally rich crop of new ones. It would be confusing and unnecessary to dwell on all these mistakes, but one of them which persisted through all three of his publications seems to have been borrowed from Chapman's paper on the foetal kangaroo (1881). This author, who examined grossly a single 14-day Macropus foetus, incorrectly described the sinus terminalis as marking the boundary between the chorion and the umbilical vesicle, not realizing that the umbilical vesicle or yolk sac forms the lining of the entire vesicle with the exception of the very small serosal spot mentioned above. While this error is not incomprehensible when based only upon gross observation, it is difficult to understand how Osborn could have failed to detect it in his microscopical slides. At any rate it was not corrected until the second half of Selenka's paper was published. Selenka's account of the development, anatomy, and significance of all the foetal membranes is remarkably accurate and complete.

In fact, I have come across only one error, and that a minor one, in his lengthy account. He described the membrane which forms the anterior wall of the body stalk between the two omphalomesenteric veins as including a double layer of ectoderm between its endodermal and mesodermal constituents (loc. cit., p. 133). This double layer of ectoderm does

not exist. The membrane referred to is composed of endoderm and somatic mesoderm, as I have already mentioned. It is illustrated in the reconstruction in figure 39. Selenka doubtless inferred the presence of the ectodermal sheets from the composition of the edges of the amniopore in stage 28, but he forgot momentarily that during stage 29 the mesoderm and its coelom push around the cranial edge of the amniopore, and separate the ectoderm at the edge of the head fold from the endoderm. The nearly closed amniopore in my reconstruction (fig. 39) shows the two layers of ectoderm which he had in mind, but they are some distance caudal to the lamella, and the latter is composed only of endoderm and mesoderm.

With this trifling exception Selenka's account of all these membranes is extraordinarily good, and it was partly with a view to giving him credit for this achievement that I chose one of his beautiful figures for a frontispiece.

The pharyngeal bursa. The reconstruction of the pharynx and foregut in figure 39 J, shows a curiously gland-like extension of the extreme anterior tip. This structure is variable in shape, number of branches, and extent of lumen. It occurs only in stage 29, which represents roughly some 8 hours of development, is always connected with the anterior end of the notochord, and has no known function. Its principal interest lies in the fact that it appears so suddenly, attains such conspicuous size, and disappears so quickly.

Selenka described it as 'die Gaumendrüse' and then again as 'die Gaumentasche.' He noticed its relation to the end of the notochord, decided that it represents the anterior notochordal canal, and that it is really not glandular in spite of appearances.

Before Selenka the following authors had described the pharyngeal bursa in man: Mayer (1840), Lushka (1860), Dursy (1869), Kölliker (1879), Froriep (1882). Of these, only the last recognized the causative part played by the notochord in this connection. Others had supposed the bursa to represent either Seesel's pouch or Rathke's pouch. As

Seesel's pouch is just another name for the anterior end of the foregut, and as the pharyngeal bursa in the opossum is located at this point, the two may be said in a sense to coincide; but that this is nothing more than a coincidence, seems clear from the fact that in man the obviously homologous bursa lies some distance caudad of Seesel's pouch. Of course, Rathke's pouch is on the opposite side of the pharyngeal membrane and has no connection with either of them.

In the same year that Selenka wrote his description, Schwaback (1887) erroneously interpreted the bursa as "a crypt connected with the formation of the pharyngeal tonsil." Selenka was correct in attributing a causal role to the notochord, but was probably mistaken in supposing that the duct of the bursa represents the notochordal canal. A recent study of this structure in man was made by G. C. Huber ('12), to whose paper I am indebted for the references to the other work on man.

The pharyngeal bursa in the opossum differs from that in man in being formed as a result of a terminal instead of a sub-terminal contact of the notochord with the pharyngeal epithelium; but I do not doubt that it has the same morphological significance.

The dorsal pancreatic diverticulum. The dorsal anlage of the pancreas arises as a long, finger-like diverticulum from the middle of the roof of what turns out to be the duodenal portion of the foregut, though it would really be a prolepsis to speak of a duodenal portion at this time as there is no visible indication of the stomach as yet. The pancreatic diverticulum is slightly caudal to that of the liver as well as being dorsal instead of ventral. It is shown in figure 39, J. In the general reconstruction it is partly hidden behind the omphalomesenteric and umbilical veins.

It is interesting that in the opossum the pancreatic anlage is present before there is any recognizable stomach rudiment. In man and in the pig it does not appear until the gastric enlargement is quite distinct. This difference may be correlated with the fact that in the opossum the liver and pancreas are both needed for the digestion and utilization of milk

at a very early time, whereas the stomach is not needed except as a reservoir and has no glands at birth.

The thyroid anlage. The thyroid in stage 29 is represented by a median ventral pocket in the floor of the pharynx between the first and second branchial pouches, but more nearly in line with the second pair (fig. 33). This location is just cranial to the truncus arteriosus. There has been a slight depression at this point since late stage 27, but it becomes quite distinct in stage 29, and in late specimens is somewhat constricted at the neck.

Miscellaneous details. The pharyngeal membrane breaks down and is resorbed during stage 29. The trachea separates from the oesophagus by the fusion and resorption of the middle portion of the lateral walls from behind forward (fig. 33).

The cranial ganglia send processes into the central nervous system. The posterior neuropore is still open on the tail bud (fig. 39, I and L). The otocyst acquires an endolymphatic duct (fig. 63, e.d.) which grows out from the dorsomedial tip of a vertical ridge on the medial wall. This point of origin is medial to the seam of closure, which is still recognizable at s.c. The first suggestion of demarcation of the seventh (geniculate) ganglion from the acoustic is recognizable in their most ventral parts, but the separation is imperfect and irregular. Dendrites from the eighth ganglion reach the otocyst. The lens placode is depressed and is in close contact with the primary optic vesicle.

The first aortic arch breaks down, leaving that portion of the ventral aorta which connected it with the second arch as the external carotid artery supplying the mandibular arch. The fifth aortic arch is still incomplete. Posterior to the level of the fifth arch a plexus from the ventral aorta extends caudad along the side of the trachea. The vitelline veins approach each other very closely in the midline behind the pancreatic diverticulum on the dorsal side of the foregut. Here they establish capillary communication with each other. In late specimens a similar situation has developed ventrad of the gut and caudad of the liver bud.

Comparative notes. With the exception of the precocious organs already listed (forelimb, lungs, etc.) the stage 29 opossum resembles very closely a 10-day rabbit (Minot's no. 562), a 3-day chick (Patten, fig. 43), a 1.4-mm. lizard (Peter's no. 74). The pancreas and allantois were not discussed in chapter IX, and need special comment.

In comparison with the corresponding organs in placental mammals, the pancreas in the opossum is precocious and the allantois is retarded. In both of these respects the opossum is more like the lizard and the chick. But in the case of the allantois the opossum is the least advanced of all the animals studied.

The placental mammal develops his allantois early and constructs from it a mechanism by which he can take advantage of his mother's pancreas, and lungs. The lizard and the chick use the allantois for respiratory purposes, but need the pancreas to enable them to utilize some of the more complicated constituents of the yolk. It is not likely that the opossum uses his pancreas in the utilization of uterine milk, but he needs it at an early stage for another reason. Even if the allantois should grow out and fuse with the serosa, it would not find any richer source of oxygen than that already found by the well-developed yolk sac circulation. The yolk sac thus serves as the source of both food and oxygen; and the allantois, which becomes completely surrounded by it, serves only as a urinary reservoir.

Apparently the need of the opossum foetus which is least well served by this arrangement is that of oxygen. And it may be that the three most precocious features of opossum development—the development of the lungs, the development of the forelimbs, and the act of parturition—are all primarily adapted to fill that need. The act of parturition carries the foetus to a rich source of oxygen, which the precocious lungs can take advantage of. But this act also necessitates the finding of a new source of food. The precocious forelimbs, as will be seen later, carry him to the new source of food. The well-developed pancreas then makes the utilization of this new food possible.

XI. THE ELEVENTH DAY. Stages 30 and 31

The first half of the eleventh day

Stage 30. External distinctive features. The secondary lumbar flexure. Bronchiolar buds. The pulmonary arteries. The liver cords. The septum transversum and the pleuro-pericardial membrane. Advances in the nervous system. Miscellaneous details.

External distinctive features. Stage 30 is distinguished from stage 29 by the secondary (convex) lumbar flexure, the

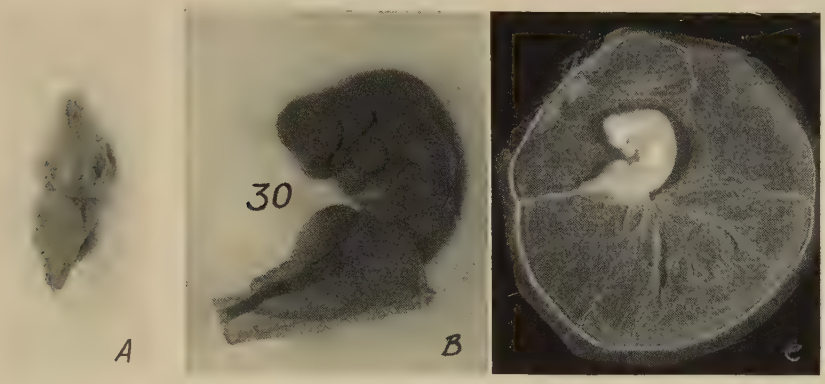


Fig. 41 Photographs of external views of stage 30. A, ventral view of 16158 after removal of all extra-embryonic membranes except the allantois and the portion of the yolk sac covering it. B, lateral view of 16160 with amnion still in place. C, lateral view of 16160 with only the non-vascular chorion removed.

flattening of the forelimb club into a paddle, and the protrusion of the allantois well beyond the body (fig. 42, I). Also for the first time there is a well-formed tail, and in most specimens the posterior neuropore has closed.

It is distinguished from stage 31 by the presence of the naso-oral groove and the absence of prominent digital ridges on the anterior limb paddle.

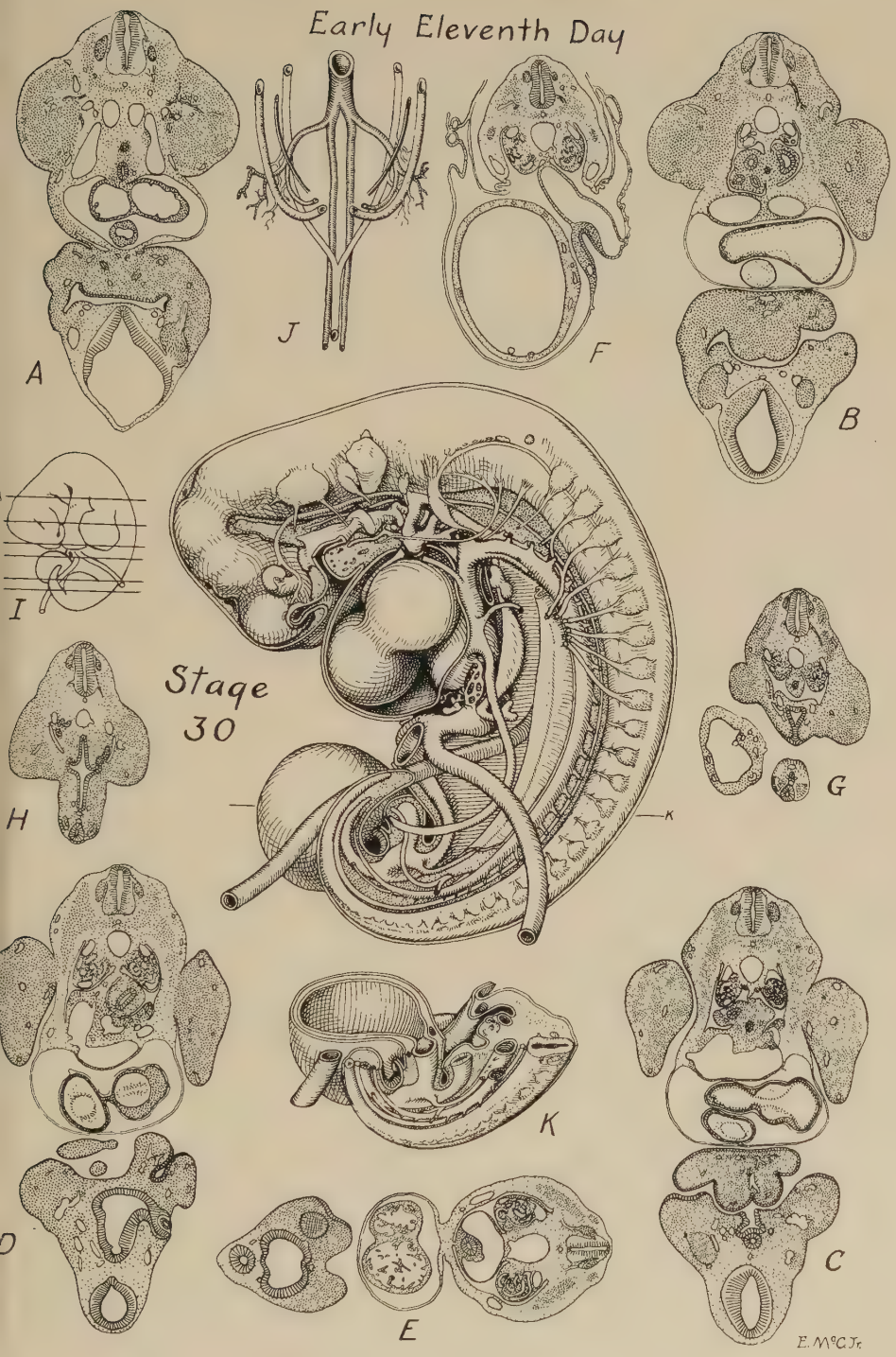
The secondary lumbar flexure. If the photographs of type specimens of stages 29 and 30 reproduced in the third normal stage plate be compared with each other, it will be seen that by far the most conspicuous difference is that caused by a

convex flexure in the lumbar region in stage 30. The heads, gill arches, and hearts of the two specimens are strikingly similar. The forelimbs differ principally in the degree of flattening. And yet the stages could never be confused. For in stage 30 the caudal end of the body has been flexed sharply in the ventral direction until it is brought up under the heart. The coiling is so extreme that the greatest linear dimension of many specimens at this time is as little as 4.0 mm. This length is less than that of any specimen since stage 23.

Linear dimension is, of course, in most animals a notably inaccurate clue to developmental stage, but this is perhaps in an exceptional degree true of the opossum. A 6-mm. opossum embryo may belong to the end of the ninth day (stage 26), the end of the tenth day (stage 29), or the end of the eleventh day (stage 31). And a 4-mm. opossum may be just acquiring its first somites (stages 22 and 23), or it may have the principal lobes of the lungs indicated (stage 30). For this reason, though average measurements are occasionally mentioned, they are given very little emphasis in this paper.

If it is true that the primary (concave) lumbar flexure was due to the rapid development of the mesonephros, which made the ventral portion of the trunk elongate more rapidly than the dorsal portion, then it is probably true that the secondary (convex) lumbar flexure is due to a spurt of growth in the lumbar spinal cord which makes the dorsal portion of the trunk elongate more rapidly than the ventral portion. This suggestion has not been verified by precise quantitative studies, but it is in harmony with the fact that the posterior limb bud first becomes conspicuous at this time, and there is a corresponding and easily visible development of the region of the spinal cord corncerned with the prospective lumbosacral plexus. In stage 29 there is a very early neural crest formation in the lumbosacral region and no sign of motor

Fig. 42 Reconstructions and sections of stage 30. The central reconstruction is from 17132. The sections and J and K are from 16158. I represents only the levels of the sections, not the size of the specimen.


Early Eleventh Day
A
J
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Stage
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E. McC. Jr.

ganglion cells or of differentiation of the three layers within the spinal cord. In stage 30 all of the sensory ganglia for the limb segments become organized out of the neural crest and develop connections both with the cord and with the limb bud; and motor ganglion cells within the cord send out axones to the limb bud. After noticing the striking similarity between the cranial, cervical, and thoracic parts of the body in stages 29 and 30, to which attention has already been called, one has only to compare section G of figure 39 with section H in figure 42 to see at once that the principal developments in stage 30 are in the posterior end of the body and especially in the spinal cord.

Bronchiolar buds. In chapter X the development of the lungs was followed from the earliest, dorsolateral, paired anlagen to the stage where they become ventral buds connected to the pharynx by means of a single, ventral tube—the trachea. In stage 30 the process of bronchiolar budding begins. This seems best described as monopodial. The original bud on each side elongates to form a stem bronchus, and as it grows caudad secondary bronchial buds arise as diverticula from its side. In stage 30 one such secondary bud has arisen on the left and two on the right. This stage corresponds to that of a 10-mm. pig or a 7-mm. human embryo as far as the lungs are concerned; but as the lungs in the opossum are very precocious this comparison does not apply to the embryo as a whole. For the less specialized organs the stage 30 opossum is better compared with a 5-mm. pig or a 10½-day (5 mm.) rabbit.

This budding goes on principally in the endodermal portion of the lung. The mesodermal blastema seems to receive quite passively the general shape of the endodermal tree as the latter expands within it; and the lobes are not well indicated on the external surface of the lungs until a later stage.

The pulmonary arteries. The origin of these arteries in the opossum has not heretofore been described. Bremer's remarks about them in his 1909 paper do not refer to the early stages. Of more interest is his description ('12) of the

aortic arches in the rabbit embryos, in which he points out that the pulmonary arteries are extensions of the ventral aortae—not outgrowths of the sixth aortic arch. In fact, they grow past the level of the sixth aortic arch before that arch is formed. This is quite definitely the case in the opossum; Federow (see Bremer, '12 b) has found it in the guinea pig; Buell ('22) found it in the chick; and Congdon ('22) found it in man. What is, therefore, usually interpreted as the ventral half of the sixth arch is really part of the ventral aorta; and the arch, which is entirely dorsal to the point of origin of the pulmonary artery, is also entirely lost in later development, no part of it ever being incorporated

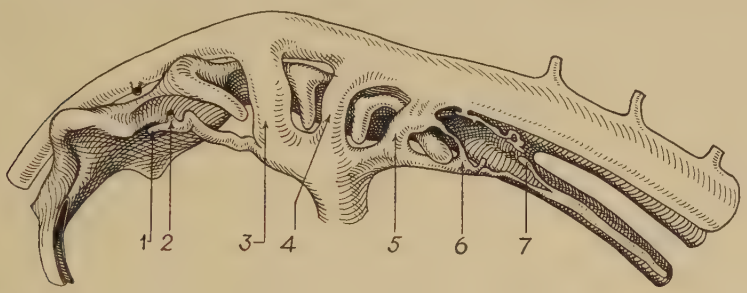


Fig. 43 Lateral reconstruction of aortic arches and pulmonary artery in stage 30, 17132.

into the pulmonary artery. Thus far, the course of events in the origin of the opossum's pulmonary artery coincides with what Bremer found in the rabbit; but in stage 30 I have found in three specimens a condition not heretofore described, and possibly of considerable significance.

Figure 43 shows a reconstruction of the aortic arches and pulmonary artery on the left side in specimen 17132. The most interesting feature of this specimen is the presence of a plexiform and not quite complete seventh aortic arch. Whether this is a normal structure I am not prepared to say with finality. The embryo is normal in every other respect. On the right side in the same specimen the rudiment is less well developed but it is represented by a ventral sprout at the

same level as the one on the left. In another specimen of the same litter (17156) there are well-developed dorsal and ventral sprouts on both sides. And finally, in one specimen (17135) from another litter there are small ventral sprouts on both sides at the proper level.

The fact that these rudiments have been found in three embryos of the same stage of development, and taken from two litters, suggests that they may be normal structures of short duration. This interpretation is strengthened by the evidence already presented that the lungs are first formed as a pair of gill pouches. For if so, the assumption that at some time in the phylogeny of the pulmonates there must have been a seventh aortic arch, is a necessary part of the theory.

I therefore follow Bremer in saying that the pulmonary arteries are certainly not derivatives of the sixth aortic arch, and that portion of what has been called the sixth below the origin of the pulmonary artery is really part of the ventral aorta. But I go one step further and suggest that beyond that point the pulmonary artery may represent still another aortic arch, the seventh or true pulmonary arch, the dorsal component of which in the embryology of most pulmonates has been lost or reduced to a vestige.

The liver cords. The originally simple and broad ventral diverticulum from the duodenum is now becoming organized into several anlagen which will have very different fates. The portion which runs most nearly craniad is the hepatic duct (fig. 42, reconstruction), and from it many solid cords of cells are growing out into the mesenchyme of the ventral mesentery. These cords ramify in the direction of the sinus venosus and between the most proximal portions of the omphalomesenteric veins, which latter respond to the presence of the cords in a remarkable way. Numerous twigs grow out from the veins and branch among the cords. In the case of the left vein this process rapidly breaks up the original channel into a complex of anastomosing sinusoids running between the cords.

A conspicuous bud running more ventrally from the original liver diverticulum is the anlage of the gall bladder (fig. 42, reconstruction, just above the omphalomesenteric vein). And finally, at the junction of the liver diverticulum and the duodenum a small ventrocaudal bud (not shown) represents the ventral pancreatic diverticulum.

The septum transversum and the pleuropericardial membrane. In the opossum the omphalomesenteric veins play no part in the formation of the septum transversum. In figure 26 it was shown how the two heart tubes and the veins which are their posterior extensions ran in the splanchnopleure before there was a foregut; how the splanchnopleure folded ventrally to form the floor of the foregut, and the heart tubes came together to form a single median heart with only a dorsal mesocardium—no ventral one. The dorsal mesocardium forks posteriorly, descends around the edges of the anterior intestinal portal (fig. 33, stage 27, d.m.l.), and continues into the extra-embryonic splanchnopleure as the attached face of the omphalomesenteric vein. In the region of the heart proper the line of attachment of the tube is dorsal; at the level of the anterior intestinal portal it spirals around the omphalomesenteric vein on its medial side; and in the extra-embryonic region it is ventral. The peritoneal cavity is thus continuous with the pericardial cavity by a passage which in stages 27, 28 and 29 is lateral to the omphalomesenteric veins at the level of the anterior intestinal portal.

The closure of this passage begins, however, back in stage 25 (fig. 25, H) when the sinus venosus expands until it touches the opposite somatopleuric wall at the level of the anterior end of the umbilical veins. In stages 26 and 27 the umbilical gains access to the sinus through the resorption of the walls at this point; and the anterior cardinal also joins in the anastomosis.

The point at which the sinus meets the somatopleure is well shown in figure 36, section D. Dorsocaudal to the sinus and on either side of the mesentery are the pleural canals—the dorsal remnants of the original wide communication between

the pericardium and the peritoneal cavity. Section C shows that these pleural canals pass the ducts of Cuvier on their medial sides. This is also particularly well shown in section B of figure 39. The central reconstruction in figure 36 shows that the pericardium also still communicates with the peritoneal cavity ventrolaterally by the pericardioperitoneal canal which passes over the omphalomesenteric vein. On each side of the body the original, single, wide communication between the anterior and posterior portions of the splanchnocoele has thus been divided in two by the developing sinus, leaving a narrow dorsal passage (the pleural canal) and a narrow ventral passage (the pericardioperitoneal canal).

The latter of these two is the first to close, and its closure is accomplished by the continued lateral and ventral expansion of the sinus venosus. Figure 39, reconstruction and sections C and D, shows the fusion of the sinus with the lateral body wall continued almost to the point of closure ventrally. In stage 30 (fig. 42) this closure is complete, and the septum transversum is thus formed by the expansion of the sinus venosus—not by the omphalomesenteric veins.

The closure of the pleural canals, however, is like the same process in other mammals. The central reconstruction shows the one on the left passing medially behind the duct of Cuvier and emerging between the duct and the lung buds. On the right (not figured) this great vein has pressed medially until its wall has fused with the mesentery and thus obliterated the pleural canal on that side. By the same process the thin slit which remains on the left side will be closed in stage 32. The membrane formed by the inward migration of the ducts of Cuvier is the pleuropericardial membrane.

The septum transversum in the opossum is thus formed by the lateral and ventral expansion of the sinus venosus. The pleuropericardial membrane, on the other hand is formed by the medial migration of the ducts of Cuvier, as in other mammals.

Advances in the nervous system. All cranial nerves (fig. 42, reconstruction) are present in stage 30 except the motor nerves of the eye. The olfactory is represented by only a few fibers from the olfactory pit to the forebrain. The optic is represented in a sense by the optic stalk, but the nerve fibers proper are only beginning to appear. The retina shows the first steps of differentiation of the cellular and fibrous layers. The gasserian ganglion has a strong connection with the hind-brain, and the three branches of the trigeminal (ophthalmic, maxillary, and mandibular) are well formed. The facial nerve has not yet developed a chorda tympani. The dendrites of the eighth nerve from the otocyst are now separable for the first time into two groups, a dorsolateral or superior group, and a ventrolateral or inferior group. The geniculate and acoustic ganglia are fused dorsally, but their ventral thirds are separated, the acoustic being medial to the geniculate. The anlage of the spiral ganglion is recognizable as a less compact bud on the ventrolateral tip of the acoustic complex. The glossopharyngeal nerve has a well-developed petrosal ganglion, but the superior ganglion is indistinct and diffuse. The vagus similarly shows a good nodosal ganglion, but the jugular accessory root ganglia are poorly formed. The spinal accessory is well developed proximally but not separated from the vagus distally. The hypoglossal can be followed all the way to the tongue anlagen. It may arise either lateral or medial to the precardinal vein. If it arises lateral, then during stage 30 it migrates through the precardinal, or the latter grows around it, so that the nerve becomes medial. It is always medial in stage 31 and later. Froriep's ganglion is present, but I have not seen any connection between it and the hypoglossal.

The first cervical nerve has no ganglion at any time. The fourth, fifth, sixth, seventh, eighth cervicals and the first thoracic form a loose and irregular fusion which is the primordium of the brachial plexus. There is no indication of a lumbosacral plexus as the nerves at that level are just beginning to grow out, and the ganglia are just becoming organized from the neural crest.

In the caudal region the neural crest is still in a primitive condition; and in very early specimens there is even a posterior neuropore in the tail.

Within the central nervous system the ependymal, mantle, and marginal layers are well differentiated in the hind-brain and cervical cord (fig. 42, sections A to E).

Larsell ('35) has found two commissures arising in the cerebellum in stage 30. The cerebellar commissure, which is the more anterior of the two, arises in connection with the trigeminal; and the lateral commissure, the more posterior, is associated with the seventh nerve. According to his interpretation these two primitive commissures form the basis of two functionally and phylogenetically different parts of the adult cerebellum—the corpus cerebelli, associated primitively with the fifth nerve, and the flocculo-nodular lobe associated with the seventh.

Miscellaneous details. In the heart the interventricular septum (E), the interatrial septum primum (A), and the endocardial cushions in the atrioventricular canal (fig. 42, C and D) are just beginning to form. Capillaries from the left part of the atrium invade the mesoderm of the lungs. From the proximal part of this plexus a single large pulmonary vein is organized during stage 30. It runs between the ducts of Cuvier and empties into the atrium just to the left of the septum primum. Some transitory capillary twigs from the anterior cardinal also ramify in the region of the trachea.

The first and second aortic arches are reduced to capillary plexuses. The third is at the height of its development. The fourth and fifth are well formed (fig. 43). It is interesting that the fifth is well developed and arises before the sixth—a primitive condition not heretofore described in a mammal.

The lens cup pinches off to form a vesicle (fig. 42, D). The optic vesicle begins to invaginate to form a cup (D). The naso-oral groove deepens very markedly (D).

The thyroid diverticulum pinches off to form a vesicle situated just in front of the fork of the ventral aortae (reconstruction). Rathke's pouch deepens and constricts, but is not yet pinched off (reconstruction).

The chondrostyle is forming around the notochord, and premuscle masses are prominent in the forelimb (fig. 42, A, B, C and D).

Figure 42, J, is a reconstruction of the wolffian duct and the blood vessels of the lumbosacral region. The wolffian duct is the very slender, black tube. The aorta and its branches are shaded with curved lines. The umbilical vein in stippled. The postcardinal vein is unshaded. It can be seen that the aorta, which became single in the lumbar region in stage 29, is in stage 30 still double at the level of origin of the common iliac arteries. The paired aortae are continued into the tail as the caudal arteries which run on either side of the tail gut. The common iliac arteries divide at the level of the limb bud into the external iliac and the hypogastric. The latter passes ventrally and cranially around the cloaca and out to the allantois. The single, median, caudal vein runs on the ventral side of the tail gut (section G), divides near the cloaca (section H) into the two postcardinals, which latter receive many small veins from the limb bud before they reach the mesonephros.

The capillary connections between the omphalomesenteric veins dorsal to the gut anterior to the liver bud, and ventral to the gut posterior to the liver bud, described in stage 29 (p. 123) have now developed into large anastomoses, so that the veins form two complete loops around the duodenum. However, the left arm of the anterior loop has already become plexiform, as described in connection with the liver cords.

A slight thickeing of the peritoneum in a longitudinal line along the medial wall of the mesonephros is the anlage of the genital ridge.

The tail gut is at the height of its development (central reconstruction).

The second half of the eleventh day

Stage 31. External distinctive features. Changes in the chorion. Changes in the venous system. The earliest lymphatic anlagen. Changes in the arterial system. Changes

in the pancreas. Pleuroperitoneal membranes. The ureteric bud. The primitive choanae. The motor nerves of the eye. Miscellaneous details.

External distinctive features. Stage 31 is distinguished from stage 30 by the loss of the naso-oral groove, the development of the frontal process, the auricular tubercles, the fusion of the mandibular and hyoid arches beneath the external auditory meatus, the appearance of prominent digital ridges on the forefoot, the enlargement of the allantois until its diameter is about one-third the greatest length of the body. It is distiguished from stage 32 by the smaller allantois, the fact that the digital anlagen on the forefoot are not yet buds; and the hind limb is still a bud, not a club, that is, its length is not appreciably greater than its width and it has no terminal enlargement.

Changes in the chorion. Up until the middle of the tenth day all of the vesicles are spherical and roll or float about freely in the cavity of the uterus. During stages 29 and 30 the animal pole of the vesicle comes to rest against the uterine mucosa. This relation is consistent in all of the vesicles, as if the area vasculosa were for some reason sticky and inclined to adhere to the mucosa. Selenka (1887, S. 128) was of the opinion that the shell membrane (which he erroneously called the 'granulosa membrane') over the area vasculosa becomes soft and adhesive at this time, and acts as the anchoring agent. At any rate, the vesicles all come to rest in this position with the area vasculosa against the mucosa and the non-vascular chorion on the free side. Osborn (1883 a and b, 1887) described the vesicles at this time as aligned in two longitudinal furrows of the uterine mucosa, but no such arrangement has been seen by any other investigator. The vesicles are usually scattered at random throughout the uterus.

After coming to rest the vesicle continues to enlarge, but the enlargement is accommodated in different ways at the attached and unattached faces of the vesicle. The attached face wrinkles into great folds which sink into corresponding

crypts of the uterine mucosa (fig. 17, also Hartman, '23, fig. 31). These folds and crypts become exceedingly complicated as development continues, but no fusion between foetal and maternal tissues ever occurs. The use of the extensive folding is only to increase the surface of contact. At any time during development the vesicles may be separated from the mucosa without a tear and without loss of either foetal or maternal blood.

The non-vascular portions of the vesicle enlarge by expanding medially into the lumen of the uterus and laterally until they meet each other. The surfaces of lateral contact

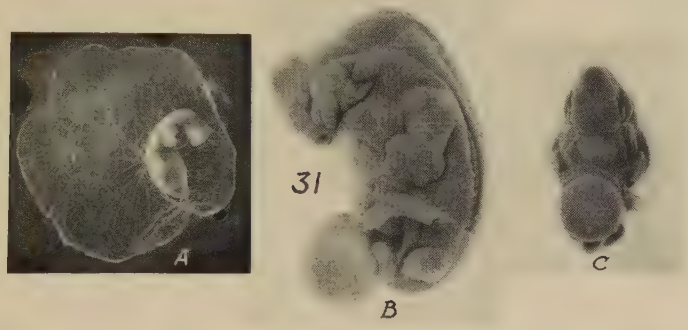


Fig. 44 Photographs of external views of stage 31. A, lateral view of 16164 photographed alive in Ringer's solution. B, lateral view of 16162 with all extra-embryonic membranes except the allantois removed. C, ventral view of same specimen.

of adjoining vesicles then fuse together so that each vesicle assumes the shape roughly indicated in figure 45, G. The vesicles thus develop non-vascular, membranous attachments to each other, but never any sort of attachment to the maternal tissues. All of the changes just recounted were accurately described by Selenka.

Changes in the venous system. The omphalomesenteric veins have enlarged enormously. The left side of the anterior loop, which was plexiform in stage 30, is altogether gone in stage 31, so that there is only a single large vein at the level of the stomach, which passes dorsad around it and up to the

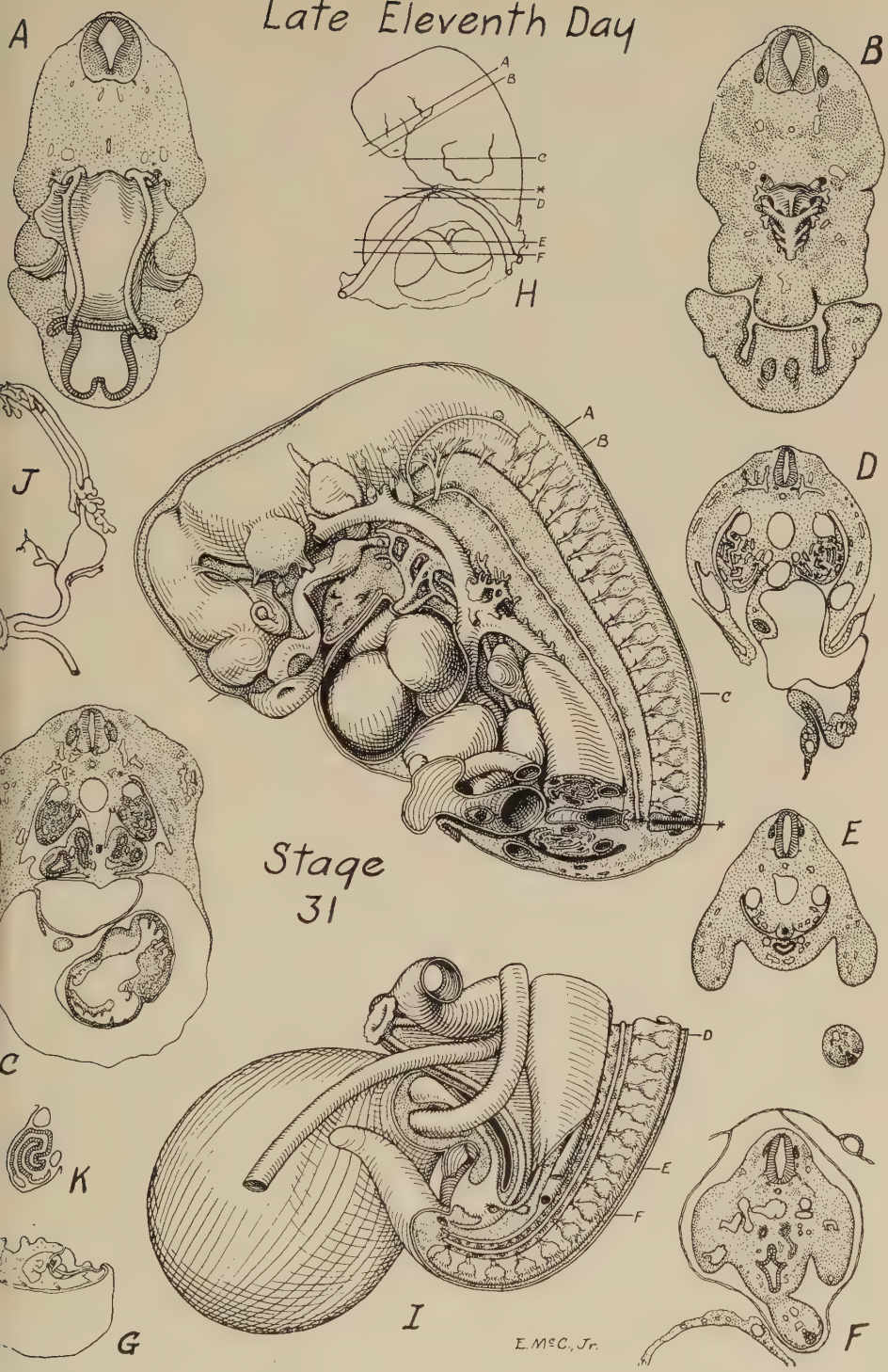
right side of the liver (fig. 45, central reconstruction). On the other hand, it is the right side of the posterior loop which has been reduced to a vestige, as seen in the cross-cut portion of the central reconstruction; and this will soon be likewise lost. Though the process is not quite complete, it is obvious that within the body the two omphalomesenteric veins are being converted into a single large vessel entering the body on the left side of the duodenum and spiralling around behind it to pass into the liver on the right. Within the liver the right omphalomesenteric is not completely destroyed or replaced by sinusoids as in placental mammals, but maintains its open channel right through to the sinus venosus up until the time of birth. It thus comes to play a part in the formation of the postcava which will be described in the next chapter.

The enlargement of the liver and the constriction of the connection between the mid-gut and the yolk sac have caused important changes in the umbilical veins. These will be discussed in more detail in the next chapter, but it should be mentioned here that the liver has grown around the anterior ends of the umbilicals (fig. 45, reconstruction) and the posterior ends are being drawn toward the midventral line (fig. 45, D).

In the mesonephros many branches from the postcardinal ramify among the tubules. In stage 31 these branches develop longitudinal anastomoses along the ventral medial border of the mesonephros. The new longitudinal channels are the subcardinals (fig. 45, D).

The primitive radial vein, which drained the ventral border of the anterior limb into the umbilical, seems to be finally lost in stage 31. In previous stages its mouth had migrated anteriorly along the umbilical until in stage 30 it emptied into the angle between this vein and the duct of Cuvier. It is possible that this same process of migration has now carried it up the duct of Cuvier to the cardinal anastomosis or promontory, in which case it is preserved as the cephalic vein of

Fig. 45 Reconstructions and sections of stage 31. The central reconstruction and I and J are from 17146. A and B are from 17147. The sections are from 16163. G shows the real size of the vesicle.


Late Eleventh Day
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E. McC., Jr.

adult anatomy, which may be seen in that position in the reconstruction in figure 45. But I have not a sufficiently closely graded series between stages 30 and 31 to establish this point, and as far as the evidence goes it seems more likely that the cephalic is a new vein.

The primitive ulnar vein has migrated along the postcardinal to the promontory. And the inferior jugular has migrated up the duct of Cuvier until it has become a tributary to the precardinal (fig. 45, reconstruction).

The earliest lymphatic anlagen. The origin, development, and adult anatomy of the lymphatic system in the opossum has been worked out in great detail by Dr. A. A. Zimmermann of the University of Illinois Medical College. This work was done partly at The Wistar Institute and partly at his own University. He very generously allowed me to read his manuscript before it went to press, and to incorporate an abstract of his embryological observations and conclusions in this book. The account of the development of the lymphatics in this and succeeding chapters is based entirely upon his work, and I am very much indebted to him for the privilege of citing it here.

The first steps in the development of the jugular lymph sacs and of the thoracic duct occur independently of each other during stage 31. In stages 27 and 28 there was formed at the angle between the pre- and post-cardinals a plexus of veins which I called the paracardinal plexus (p. 110). In stage 31 this plexus is divided into dorsomedial and dorsolateral portions. The former is the more cranial of the two. It is lateral to the intersegmental veins 1 to 5, and medial to the cervical nerves 4 and 5. Anteriorly it communicates with the first intersegmental vein and posteriorly with the jugular promontory near the mouth of the external jugular (jugulocaphalic) vein. The dorsolateral portion is medial to the blastema of the scapula and lateral to the cervical nerves 3 to 7. It communicates with the jugular promontory dorsolateral to the common stem of the third and fourth intersegmental veins (fig. 45). This connection coincides with the mouth of the primitive ulnar vein. It also communicates by a small venule with the dorsomedial portion described above.

Both of these venous plexuses show signs of degeneration in stage 31 (16163), but particularly the dorsomedial or more cranial of the two. The channels are becoming sinusoidal or sacculated, and the endothelium is breaking down. Perivenous spaces form in the mesenchyme immediately around these veins and develop an independent mesenchymal endothelial lining before the endothelium of the veins breaks down (fig. 48). These perivenous spaces are lymphatic anlagen. They do not form in the mesenchyme surrounding the functional or permanent vessels in the same region, but are consistently found around all venules which are to be replaced by lymphatic channels. When the endothelium of the vein thus surrounded breaks down, the blood is liberated into the primitive lymphatics, which are thus at first hemophoric.

At the same time that these changes are occurring in the region dorsolateral to the confluence of the cardinal veins, another set of changes is in process in the mesenchyme ventral to the aorta in the lower cervical and upper thoracic region. Here the mesenchyme is becoming distinctly vacuolated as if the intercellular fluid is increasing. As the mesenchymal clefts enlarge, the cells lining the spaces assume endothelial characteristics. At first the lymphatic anlagen thus formed are isolated, but later they run together to form a plexus which is continuous with other mesenchymal spaces of perivascular origin like those which coalesce to form the jugular sacs.

The thoracic duct thus is derived from two sorts of anlagen —some which form altogether independently of veins, and others which form around degenerating venules. In all cases, however, the anlagen develop their own independent endothelium from mesenchyme.

Changes in the arterial system. Among the aortic arches the principal flow of blood seems to be shifting progressively caudad, so that now the fourth arch has become the largest of the group (fig. 45). The fifth is longer and slenderer than in stage 30, but it still has a larger caliber than it ever attains in most mammals. The endocardial ridges of the conus are causing a partition to be formed between the base of the fifth arch and the ventral aorta, pushing the fifth forward with the fourth. The sixth has enlarged until it is about the same size as the ventral aorta, which latter correspondingly looks

like the ventral half of the arch, and the impression is thus produced that the pulmonary artery is a branch from the middle of the sixth arch. Actually the sixth is entirely dorsal to the origin of the pulmonary, as pointed out in connection with stage 30.

The first seven intersegmental arteries become plexiform and develop longitudinal anastomoses with each other dorsal to the aorta. The first six lose their connections with the aorta, and the anastomoses plus the remnants of the intersegmentals form the vertebral arteries in stage 31. The seventh intersegmental artery, from which the vertebral now appears to arise, is at the level of the limb bud and becomes enlarged to form the subclavian artery.

Changes in the pancreas. In stage 31 the dorsal pancreatic diverticulum loses its connection with the duodenum, so that the anlage of the duct of Santorini is lost, and correspondingly no accessory duct appears in the adult. The ventral pancreatic diverticulum has migrated away from the angle where it originally formed, and now appears as a branch of the hepatic diverticulum or ductus choledochus. It is not yet in contact with the dorsal pancreas, which is thus completely detached from the digestive tract at this time. With the exception of the absence of the duct of Santorini this stage is like that of a 10-mm. human embryo. In figure 45, J, the ventral pancreatic bud is not well shown, as it is almost completely hidden by the duodenum.

Pleuroperitoneal membranes. The anterior end of the pleuriperitoneal cavity is roughly pyramidal in shape. In cross section it appears triangular with the mesonephros at the apex of the triangle, the lung at the medioventral corner, and a new fold of mesodermal tissue at the lateroventral corner (fig. 45, C). If traced anteriorly this fold, the pleuroperitoneal membrane, can be followed to the anterior apex of the pyramid where it merges with the mesonephric fold. In the middle sections the inner edge of the fold is directed toward the interval between the lungs and the mesonephros; and at its extreme caudal end it is continuous with the

coronary appendage of the liver (also called the dorsal pillar of the diaphragm). The part that these folds play in separating the pleural cavity from the peritoneal cavity will be described in connection with stage 34.

The ureteric bud. About 48 hours before birth the first rudiment of the ureter is just forming as a very small bud from the wolffian duct near the place where it empties into the cloaca (fig. 45, I and F). Even at this early stage the mesenchyme immediately around the bud shows a definite condensation, the metanephric blastema. This mesenchyme is part of the same nephrogenic mass in which the mesonephric tubules are formed. At first (16174) it is situated right in the posterior end of the mesonephros. Later (16173) it migrates medially and dorsally out of it.

The primitive choanae. The disappearance of the naso-oral groove noted in connection with the external distinctive features of stage 31, is caused by the overgrowth and fusion of its edges, converting the groove into a tube connecting the roof of the mouth with the surface of the snout (fig. 45, reconstruction and section B). This involves the fusion of the median and lateral nasal processes and the maxillary process. This direct conversion of the oral end of the naso-oral groove into the primitive choana is characteristic of the Sauropsida, but is not supposed to occur in mammals.

The motor nerves of the eye. With the appearance in stage 31 of the third, fourth, and sixth nerves the list of cranial nerves is complete. The oculomotor develops rapidly, and can be followed all the way to the eye, but the trochlearis and the abducens are extremely rudimentary at this time.

Miscellaneous details. The stomach is noticeably enlarged. Rathke's pouch has pinched off from the oral cavity. Sympathetic trunks have formed in the thoracic region. The cerebral hemispheres are enlarged. Periotic mesenchyme has condensed slightly as the first stage in the formation of the otic capsule. The allantois is as big as the head and hangs free in a pocket of the yolk sac. It is probably already functioning as an urinary reservoir, for the wolffian duct became

patent into the cloaca in stage 30, and the mesonephros gives every indication of being functional in stage 31. Somites are still forming in the tail. Auricular appendages are forming on the heart.

XII. THE TWELFTH DAY. Stages 32 and 33

The first half of the twelfth day

Stage 32. External distinctive features. The veins of the liver and mesonephros. The thoracic duct and the jugular lymph sacs. The adrenal cortex. The foramen ovale. The subintestinal vein. Changes in the branchial pouches. Changes in the digestive tract. Miscellaneous details.

Fig. 46 Photographs of external views of stage 32. A shows a ventral view of 16173 with all extra-embryonic membranes removed. B shows a lateral view of the same specimen. C shows 16174 with the allantois still attached and partly dissected.

External distinctive features. Stage 32 is distinguished from stage 31 by having the diameter of the allantois approximately two-thirds the length of the body (instead of one-third); by the hindlimb being a club instead of a bud; by having definite digital buds on the forelimb instead of ridges; by having the ventral thoracic wall opaque instead of transparent; and by having the interval between the first and sixth auricular tubercles very much enlarged. It is distinguished from stage 33 by the absence of the upper eyelid folds, the smaller size of the allantois, and by the hindlimb being a club, not a paddle.

The veins of the liver and mesonephros. In connection with stages 30 and 31 I have described the conversion of the original paired omphalomesenteric veins within the body into a single vein starting on the left of the gut (orginal left omphalomesenteric) then swinging dorsally around behind the gut (dorsal anastomosis between the omphalomesenterics) and finally entering the liver on the right side (original right omphalomesenteric). It was also mentioned that within the liver the right omphalomesenteric, though it develops numerous branches in all directions, retains its principal channel intact. Both right and left omphalomesenterics can be recognized at their anterior extremities just before they join to empty into the sinus, but posterior to this point only the right can be followed continuously through the liver.

It was also mentioned that during stage 31 the folding in of the lateral body wall, which is converting the original wide communication between the mid-gut and the yolk sac into the narrow yolk stalk, causes the anterior third of the umbilical veins to swing ventrally and approach each other near the middle line. Here they become involved in the growing liver and during stage 32 are broken up into sinusoids just as the hepatic portion of the left omphalomesenteric was before them. Further changes in these vessels will be described in connection with stages 33 and 34.

The liver is enlarging very rapidly in stage 32 and on the right side, where there is no stomach in the way, the so-called right coronary appendage of the liver expands dorsad against the mesonephros and the dorsal mesentery. Small vessels from the liver and from the subcardinal vein invade the mesentery, unite, and enlarge to form a conspicuous anastomosis which leads directly from the subcardinal to the right omphalomesenteric vein within the liver (fig. 47, C).

At the same time another important change is taking place in the posterior portion of the subcardinals. From the time of their origin the subcardinals have always communicated with the postcardinals by means of numerous sinusoids running through the mesonephros (the renal portal system). Now in stage 32 two of these anastomoses at the level shown

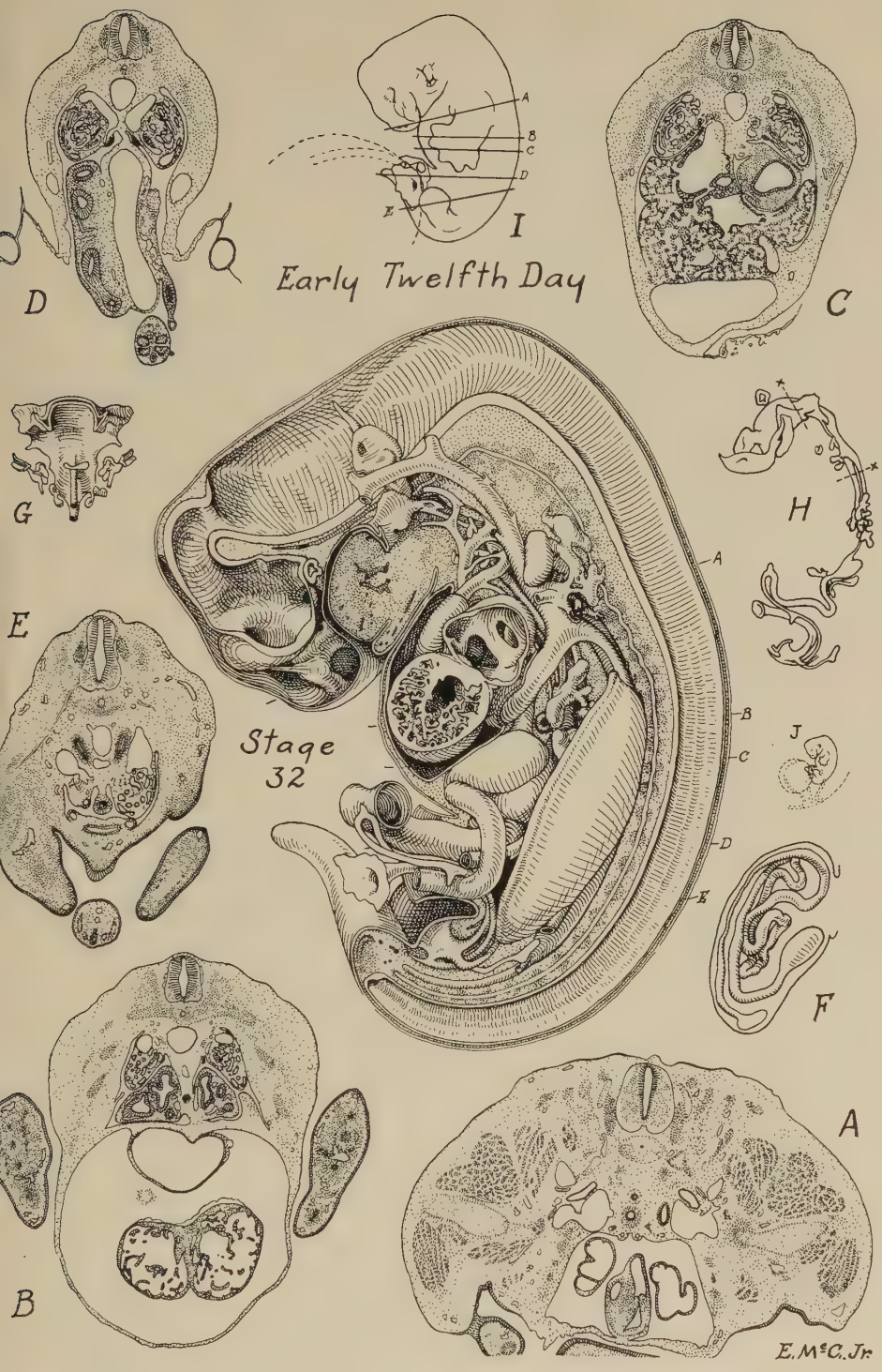
in section D become very large, and the principal flow of blood from the posterior part of the body is diverted ventrad at this point into the subcardinals. These changes constitute the first steps in the formation of the postcava, which will be discussed further in connection with stage 33.

The thoracic duct and the jugular lymph sacs. It was mentioned in the previous section that the earliest anlagen of the thoracic duct are mesenchymal spaces not associated with veins, but that these later coalesce with a second set which are formed around collapsing venules. Zimmermann has pointed out that the first perivascular lymphatic anlagen of the thoracic duct arise in stage 32 (16174 and 17127). They are located in the mediastinum between the levels of the second and seventh thoracic vertebrae. "Caudal to these levels," he says, "there are no signs of thoracic duct anlagen in Normal Stage 32. Cephalad, only the ill-defined mesenchymal clefts of the preaortic mesoderm are found."

The degenerating venules around which these first perivascular thoracic duct anlagen form, are tributaries of the thoracic portion of the postcardinal veins. Almost immediately after they begin to form, these peri-intimal spaces run together and establish a lymphatic plexus. The fusion of the separate anlagen occurs first at the levels of the fifth and sixth thoracic segments, and later spreads from this point in both directions longitudinally. As the process extends cephalad the mesenchymal clefts formed in stage 31 are met and incorporated in the lymphatic plexus. Between the most anterior of these and the anlagen of the jugular lymph sac there is still a long gap in which no lymphatic anlagen are found.

The jugular lymph sacs are at a similar stage of development. The remnants of the degenerating paracardinal venous plexus have pinched off and are completely detached from the venous system. The perivascular lymphatic anlagen are coalescing to form a continuous lymphatic plexus not in com-

Fig. 47 Reconstructions and sections from stage 32. The central reconstruction is from 16173 except for the membranous labyrinth which is added from 17127. H and G are from a wax model made by Dr. C. H. Heuser and now in The Wistar Institute. A, E and F are from 17127. The other sections are from 16174. J shows the real size of the embryo and allantois.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E. McC. Jr.

munication with the venous system at all. The endothelium of the lymphatics is of mesenchymal origin and is not derived from, or until a later stage even connected with, the venous endothelium.

Heuser's note ('19) about the anatomy of a specimen of this stage referred to the jugular lymph sac as having "become transformed into a much-divided structure with smaller and larger spaces." Instead of the sac having become divided into the several elements referred to, the latter are really in process of uniting to form the sac. His further note that "In an injected embryo of the same litter the lymph sac received no ink, although the injection of the blood vessels is complete" is in harmony with Zimmermann's observations that the jugular lymph sac has not yet acquired communication with the venous system.

Though the lymphatic system has only begun to form at this time, the method of origin thus far described will apply equally well to the later stages. In far the majority of cases the original lymphatic anlagen are spaces which form around collapsing venules. Such a peri-intimal space acquires an endothelium of its own (fig. 48) before that of the vein has disintegrated. When the venous endothelium breaks down, blood is liberated into the lymphatic which is thus for a while hemophoric. The separate lymphatic anlagen coalesce to form a plexus which secondarily acquires communication with the venous system.

The fact that the lymphatics usually, though not invariably, form around collapsing venules, accounts for the frequently recorded observation that they nearly always follow the path of a pre-existing venous plexus. In all cases they are of mesenchymal origin, and they never derive their endothelium from veins.

The adrenal cortex. In late specimens of stage 31 and in all specimens of stage 32 cords of mesodermal cells proliferate from the peritoneum near the mesentery at the level of the posterior end of the lungs and caudad as far as the region of the stomach. These cords of cells, the anlage of the cortical

part of the adrenal gland, form fairly compact masses just dorsal to the subcardinal veins (fig. 47, C).

The foramen ovale. In stage 32 (fig. 47, reconstruction) the septum primum has descended some two-thirds of the way toward the atrioventricular canal, and has become secon-

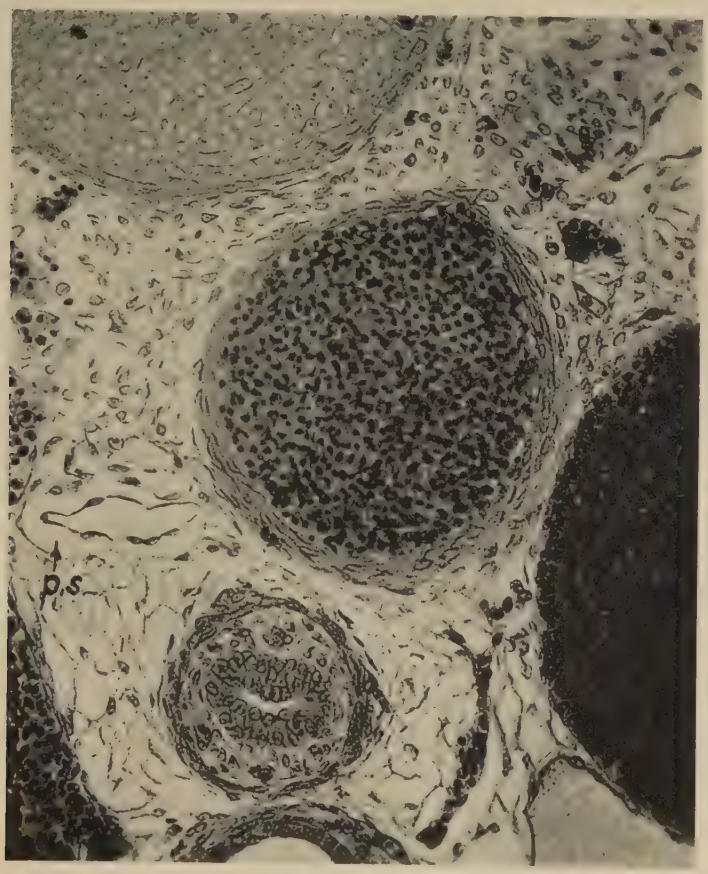

Fig. 48 Photograph by Dr. A. A. Zimmermann showing a peri-intimal space with its own endothelium forming while that of the venule is still intact.

darily perforated by one or more foramina. When there are more than one, which is always the case in later stages, usually one of the more ventral ones is decidedly larger than the others (fig. 51). This multiple condition is a primitive feature not found in placental mammals.

The subintestinal vein. Senior ('25) was the first to observe that the subintestinal vessel in the opossum is a vein, and he pointed out that in this respect the opossum is like some of the lower vertebrates, and unlike the placental mammals, in which the vessel is always an artery. Kimball ('28) made a comprehensive study of the vessel, and concluded that it is a vein in all chordates in which there is no allantoic placenta, and that it acquires new connections and becomes converted into an artery when an allantoic placenta is formed. This seems well established by her extensive investigation.

In early stages of the development of the allantois in the opossum (e.g., stage 30, 16160, slide 4) two very small veins on its dorsal wall drain caudally into the subintestinal vein and thence forward into the omphalomesenteric. I shall refer to these as the primitive allantoic veins to distinguish them from the much larger and more important umbilical veins which become the definitive veins of the allantois. As the allantois is a diverticulum from the hind-gut, it is natural that it should carry with it some of the latter's circulatory apparatus, but these vessels never become large enough to be of much functional significance, and they disappear altogether by stage 31.

In stage 32 (16174, 16173) the subintestinal vein thus has no connection with the allantois. Its posterior end is very plexiform and communicates by a few small branches with the postcardinals, but its anterior portion is still well developed (fig. 47, D) and drains forward into the omphalomesenteric. This is the stage which both Senior and Kimball studied.

Changes in the branchial pouches. The third pair of pouches become detached from the pharynx in early stage 32 (16174, fig. 47, G). At this time no migration has occurred, so all the elements are readily identifiable.

In late stage 32 (17127) all of the gill pouches are detached and considerable shifting has occurred so that determination of the origin of the different elements is impossible without a large number of closely graded specimens. I have only three

specimens of stage 32, which is not enough, as the shifting seems to take place quite rapidly.

Accordingly I can only say that if the shifting is in general similar to that which occurs in other animals (and I have no reason to think it is not) then the situation at the end of stage 32 is as follows: The thyroid has migrated caudally until it is just ventral to the ultimobranchials (fifth pair). The third pair, including both dorsal and ventral components, has migrated most caudad of all so that it lies posterior to the aortic arches and just anterior to the pericardium. The fourth pair lies between the thyroid and the third pair. The only peculiar detail here is that the dorsal (parathyroid) elements of the third and fourth complexes migrate caudally with the ventral (thymus) components instead of remaining near the thyroid.

Changes in the digestive tract. The greater curvature of the stomach (original dorsal border) is bulging very prominently to the left. The yolk stalk has pinched off leaving a temporary hollow diverticulum at the level of the union of right and left omphalomesenteric veins (fig. 47, reconstruction). A new outgrowth from the intestine, the caecal diverticulum, can be seen where the umbilical vein curves to run out into the allantois. This diverticulum was noticed, but not identified, by Heuser ('19).

Miscellaneous details. The otocyst has acquired its first semicircular canal. A nasolachrymal chord has grown down from the anterior corner of the eye toward the nasal epithelium, but has not yet reached it. The turbinals are developing.

The mesenchymatous anlagen of all cartilaginous bones except some of the caudal vertebrae are present. The vertebral anlagen become less and less differentiated in caudal progression, until in the tail itself there are still somites in early stages of differentiation. There are about sixteen of these in the tail. Chondrification is beginning in all cartilage bones except the periotics and some of the caudal vertebrae. No membrane bones except the clavicle are present.

The six rhombic grooves are quite conspicuous in the floor of the hind-brain. The lower eyelid fold is recognizable. Epitrichial cells are making their first appearance.

During stage 32 mesoderm invades the interval between the pericardium and the ectoderm so that the ventral and lateral thoracic walls become opaque. Within this mesenchyme the anlagen of sternal bars, ribs, and coracoid become differentiated.

Heuser studied two of The Wistar Institute specimens of this stage (16173 and 16174) and the following notes are taken from his abstract ('19): "The ganglion nodosum is fused with the cervical sinus, and there arises from the ganglion a cord of cells which, with a strand from the thymus, makes another connection with the sinus. The hypoglossus is embedded in the nodosum, but does not touch the thymus. The superior laryngeal nerve is distinct: it extends from the mesial border of the cord above referred to and runs between the thymus and the parathyroid." All of these points are illustrated in figure 47, G, which is taken from his wax model.

In the heart there is a large septum spurium and no septum secundum. The foramen ovale has already been described. The interventricular septum is forming but is not large (fig. 47, B). The pulmonary trunk has twisted about the aorta until it leaves the conus on the left instead of the posterior side.

The second half of the twelfth day

Stage 33. External distinctive features. The veins of the liver and the postcava. The septum primum. The jugular lymph sacs. The thoracic supracardinal (azygos) veins. The paravertebral lymphatics and the thoracic duct. The migration of the gill pouch derivatives. The allantois at its height. Miscellaneous details.

External distinctive features. Stage 33 is distinguished from stage 32 by the following details: upper and lower eyelid folds are quite distinct; there are real digits, not just digital buds, on the forefoot; the beginning of the pontine flexure

has shortened the distance between the cephalic and cervical flexures and lifted the head somewhat away from the chest; the mouth is sometimes open and the tongue protruding; the allantois has attained its maximal size with a diameter equal to or greater than the body length (fig. 49, C). It is distinguished from stage 34 by the fact that the epitrichium has not yet covered the eyes, ears, or mouth; and there are no claws on the forefeet or digital ridges on the hind feet.

The veins of the liver and the postcava. In stage 32 the right subcardinal attained communication with the right omphalomesenteric vein in the liver by way of the dorsal mesentery and the right coronary appendage of the liver.

Fig. 49 Photographs of external views of stage 33. A, ventral view of 16176. B. lateral view of 16176. C, lateral view of 16177 showing maximal size of allantois.

This affords a very direct path for the blood from the posterior part of the body to the right atrium, and in stage 33 this channel enlarges until it becomes the most conspicuous vein in the body (fig. 50, postcava). The posterior portion of the omphalomesenteric appears now as a tributary to it. This infrahepatic portion of the omphalomesenteric receives the subintestinal vein, and is the anlage of the hepatic portal; but as it opens directly into the postcava, it is not yet, strictly speaking, a portal, taking portal to mean a venous channel interrupted by capillaries.

The two umbilical veins have fused into a single vessel from the umbilicus craniad to the liver. Within the liver a new vessel of hepatic origin, the ductus venosus, drains this common umbilical into the postcava (right omphalomesenteric portion).

The left subcardinal anterior to the anastomosis is a very small vessel which will be preserved only on account of its

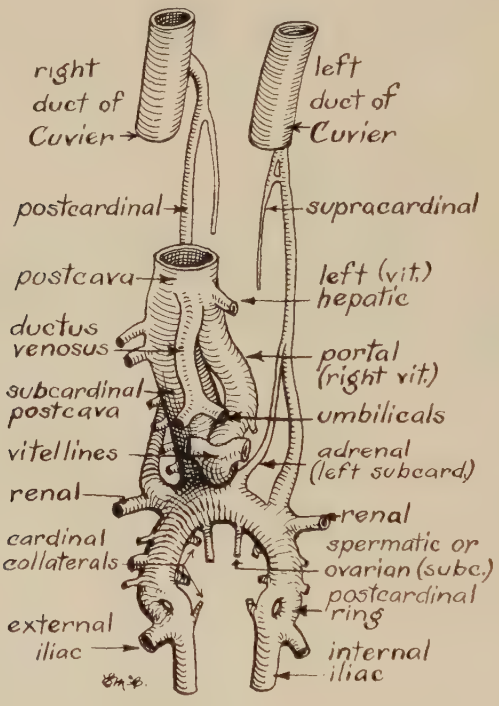

Fig. 50 Reconstruction of principal veins in stage 33 (slightly modified from McClure, '06).

association with the developing left adrenal gland. The right adrenal gland is drained by a branch directly from the postcava which is at this level the right subcardinal.

The fact that the right omphalomesenteric vein does not become broken up into sinusoids as the left does, results in its being transformed into most of the hepatic portion of the

postcava. The branches from it which extend through the coronary appendage and dorsal mesentery to communicate with similar branches from the right subcardinal, constitute a new vessel, the vein of the caval mesentery, but this contributes a relatively small part of the definitive postcava connecting the omphalomesenteric and subcardinal portions. From the liver back to the level of the metanephroi the postcava is the original right subcardinal. At the level of the kidneys it is the subcardinal anastomosis. Posterior to this point up until birth the only important channels are the subcardinal-postcardinal anastomoses and the postcardinals; for the subcardinals posterior to the anastomosis are not well developed, and there are no supracardinals.

The posterior portion of the postcardinals originally passed the common iliac artery on its dorsal side (fig. 42, J). In stage 33 it has sent sprouts around the artery which fuse to form a circumarterial ring (figs. 50 and 52), which was first described by McClure ('06). At this time another set of vessels is developing ventral to the aorta. These cardinal collateral veins, as McClure named them, develop posterior to the large subcardinal-postcardinal anastomosis from the mesonephric venous sinusoids which connect the postcardinals and subcardinals. They later become organized into two channels which replace first the subcardinals and later even the postcardinals in this region. In the formation of the postrenal division of the postcava they play the part which in placental mammals is played by the supracardinals; but unlike the latter the cardinal collaterals are entirely ventral to the aorta. In accordance with Butler's generalization ('27) the high development of the mesonephros in the opossum prevents the postcardinal from taking any part in the formation of the postcava; but why the vessels which replace it should be unlike those of all eutherians is not explained.

Posteriorly the cardinal collaterals are connected with the venous ring around the common iliac arteries already described. And about 1 week after birth when the postcardinals in this region degenerate, the circumarterial rings discharge

all of their blood into the cardinal collaterals. McClure has shown that either the right or the left or both cardinal collaterals can persist, and either the dorsal or the ventral or both halves of the circumarterial ring can persist. By various combinations of these possible routes several types of postcava are commonly formed in the opossum. In the adult the postcava posterior to the spermatic veins may be single or double with about equal frequency. It may also pass ventrad, dorsad, or both, around the common iliac artery. All of these types must be considered 'normal' (McClure, '03). The opossum is simply plastic in this respect, and has not settled upon a single 'normal' method of forming the postcava.

Aside from the plasticity, the two principal peculiarities about the opossum's postcava are: 1) that the hepatic portion is formed almost entirely from a persistent right omphalomesenteric vein, instead of from hepatic sinusoids; and 2) that the postrenal portion is formed from the unique cardinal collateral veins instead of from the postcardinals or supracardinals.

The septum primum. The interatrial septum has grown ventrad until it has reached the endocardial cushions. In stage 33 it is always cribriform, and the older the specimen the finer the mesh (fig. 51). Since in the previous stage there is sometimes only a single foramen (fig. 47, central reconstruction), and in later stages there are always many, and the later the stage the smaller the individual foramina, it seems necessary to assume that the muscular slips grow across and subdivide the original aperture. This process carried to its limit is presumably the mechanism of closure.

No septum secundum forms. At birth there are still a few small perforations through the septum primum, but they become gradually grown over and a few days after birth the two atria are separated by an imperforate partition.

In having no septum secundum, and in having many small interatrial foramina instead of a single large one, the opossum is similar to the chick and unlike the placental mammals. It has been known at least as far back as 1868 (see reference to

Owen in Cunningham, 1882, p. 150) that the adult marsupial heart has no fossa ovalis. I believe, however, that the explanation of this fact, namely, that it is due to the total lack of a septum secundum in the embryo and the presence of an avian type of cribriform septum primum, has not hitherto been recorded.

Evidently the equalization of pressure on the two sides of the septum due to the increased circulation of blood through the lungs after respiration begins, has nothing to do with the

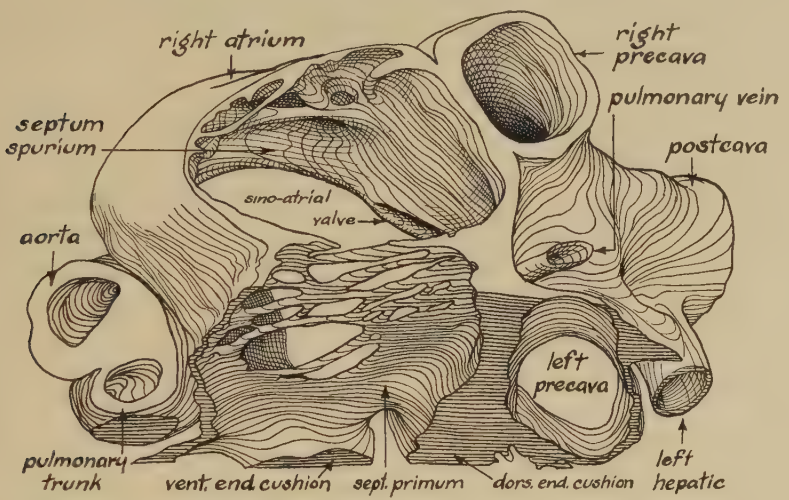

Fig. 51 Reconstruction of dorsal half of heart from 17125 showing the cribriform septum primum and the absence of any septum secundum.

closure of the interatrial foramina in the opossum, as the process of closure is almost complete before respiration begins. Also in prerespiratory stages there is no tendency for the septum to bulge into the left atrium. Figure 51 shows the foramina wide open, but the septum flat. If the pressure is unequal, then the septum is rigid enough to withstand it. Under these circumstances there is no way in which equalization of pressure could close these foramina. They must be closed by growth and fusion of the muscular bands.

The jugular lymph sacs. During stage 33 the many, small, separate, lymphatic spaces formed in the paracardinal region fuse together to form a continuous, though somewhat complicated, jugular lymph sac; and this sac acquires a connection with the venous system by means of a bicuspid valve located on the ventral side of the junction of the internal jugular (anterior cardinal) and external jugular (jugulocephalic) veins.

The anatomy of the jugular sac will be treated in detail in Doctor Zimmermann's monograph, so only a few words are necessary here. It is composed of two principal divisions: 1) a cephalic division (fig. 52), which is penetrated by the levator scapulae ventralis muscle (also called the m. atlantoscapularis anticus) and branches from the third and fourth cervical nerves; and 2) an axillary portion lying mediad of the scapula, which is derived from the dorsolateral lymphatics previously described as paralleling the dorsal ulnar vein. Two small extensions or diverticuli from the cephalic portion should be mentioned on account of their prospective significance. One of these, the medial jugular extension, runs mediad from the slender, lower segment of the cephalic portion. It represents part of the jugular arch which will later connect the jugular sac with the thoracic duct. The other, the intermediate caudal extension, runs laterad from the same slender neck of the cephalic portion of the jugular sac, and will later become the cervical approach of the lymphatic channel which parallels the external mammary artery.

The thoracic supracardinal (azygos) veins. The thoracic intersegmental veins from the postcardinal at first (i.e., up until stage 32) lie consistently lateral to the sympathetic trunks. During stage 32 supplementary, minor channels pass around the nerves on the medial side and there is thus a double drainage into the postcardinals. In stage 33 longitudinal anastomoses form between the medial radices of the intersegmentals, the lateral radices drop out, and the postcardinal trunk ahead of the mesonephros degenerates. The longitudinal vessels thus left on the medial side of the sympathetic trunks and draining into the common cardinal, are the supracardinal or azygos veins.

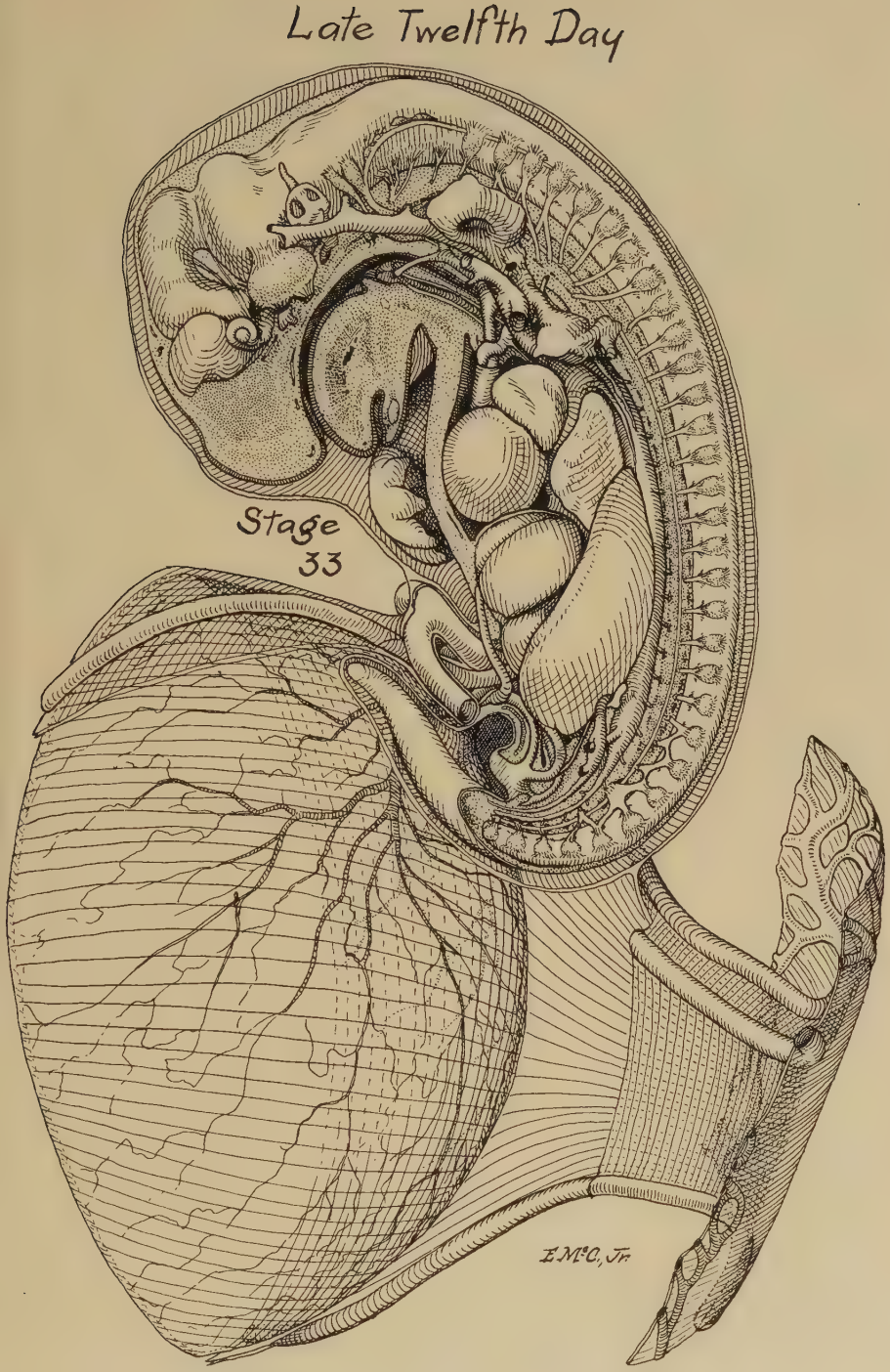

Fig. 52 Reconstruction of stage 33 (17126). Compare with frontispiece.

From the beginning the right and left supracardinals differ in a few minor respects. The right vessel gains access to the common cardinal through a short segment of the postcardinal, which segment is thus preserved and incorporated into the right azygos vein. The left supracardinal, on the other hand, drains directly into the common cardinal, and the entire thoracic portion of the postcardinal degenerates. Both supracardinals are sometimes preserved in the adult opossum, but the left is always the better developed of the two.

The paravertebral lymphatics and the thoracic duct. In connection with the dropping out of the lateral radices of the original intersegmental tributaries to the postcardinal, Zimmermann has pointed out that the degenerating vessels go through a history quite like that of the paracardinal plexus. That is to say, they become detached from the stem veins, break up into sacculated, discontinuous, remnants surrounded by the usual peri-intimal spaces. These spaces in the usual way develop an endothelial lining, sprout longitudinally, and coalesce to form a lymphatic channel running longitudinally on the lateral side of the sympathetic trunk. This new channel he calls the paravertebral lymphatic.

At the time of birth (stage 35, 17173) the left paravertebral lymphatic has grown craniad around the dorsal side of the left azygos vein and attained communication with the left thoracic duct. The right at this time is still independent. Both vessels receive the intercostal lymphatics which are apparently derived as sprouts from them.

When in stage 33 the supracardinals replace the postcardinals in the thoracic region, ventromedial sprouts from them into the mediastinum serve, as in the case of the similar ones from the postcardinals, as the skeleton around which more anlagen of the thoracic duct form. The thoracic duct thus grows craniad. Its most cranial anlagen in stage 33 are located at the level of the junction of the common cardinal with the sinus venosus, and instead of being grouped together in the midsagittal plane, they tend to separate into two bilaterally symmetrical channels. This foreshadows the fact that in the adult opossum the thoracic duct branches symmetrically at its anterior end to empty into both jugular lymph sacs.

Migration of the gill pouch derivatives. In early stage 32 (16174) the third gill pouches become detached from the pharynx (fig. 47, G). In late stage 32 and in stage 33 the

fourth and fifth gill pouches are also detached and considerable migration has occurred. As there is no histological differentiation between the derivatives of the different pouches at this time, identification becomes complicated. This much can be established with certainty: 1) the thyroid has migrated caudad until it is just ventral to the ultimobranchials (fifth pair); and 2) the third and fourth pairs, including both dorsal and ventral components, have migrated further caudad. The fact that the dorsal (parathyroid) elements of the third and fourth descend with the ventral (thymus) elements into the mediastinum is clear. The only point about which there is any uncertainty is which are the third derivatives and which are the fourth. As both sets have a similar history the question is not important. If, as in other mammals, the third gill pouches migrate most caudad of all, then the four epithelial bodies caudal to the aortic arches and just cranial to the pericardium are probably the two ventral and the two dorsal elements of the third pair. Correspondingly, the four units situated around the common carotid arteries just anterior to the fourth aortic arches are probably the dorsal and ventral elements of the fourth pair. At any rate, in later stages all eight derivatives of the third and fourth branchial pouches come together in the mediastinum to form the thymus and the parathyroids.

It has been known for some time that in the adult opossum no parathyroids can be found in or near the thyroids. This is due to the fact just mentioned, that all four parathyroids become incorporated into the thymus in the mediastinum.

During stage 33 the ultimobranchials come into contact with the dorsal walls of the wings of the thyroid (17151) and fuse with the latter just as the isthmus of the thyroid disappears. These are the so-called 'lateral thyroids' of older authors. Whether they contribute any real thyroid tissue or not, I am unable to say, as they seem to become completely lost in the gland before differentiation of colloid vesicles occurs.

The allantois at its height. The principal facts in the development of the allantois have been described in connection

with the discussion of the extra-embryonic membranes in stage 29. But as it reaches it peak of development in stage 33, a few more words about it are necessary at this point.

Figure 49, C, shows how large the allantois becomes at its maximum. In the specimen here photographed the chorion and amnion have been dissected away. The frontispiece and also figure 52 show the relations of the allantois to these other extra-embryonic membranes. Looking at the frontispiece it is easy to visualize the complete vesicle, and to see that the embryo proper and the allantois are in a pocket hanging into and completely surrounded by the endodermal yolk sac, which lines the inside of the entire vesicle.

In this position deep inside the yolk sac the allantoic vessels cannot serve in a respiratory capacity. This function is left entirely to the vitelline vessels, which are permanently superficial. Accordingly, the allantoic vessels do not develop beyound the extent necessary to serve the immediate needs of the thin-walled allantois itself. As the mesonephroi have been functional for at least 24 hours (probably as early as stage 30), and as no mechanism is provided for the transfer of the urine to the maternal blood, the urine has to pile up in the allantois, which correspondingly enlarges to accommodate it.

Finally, in stage 34 the cloacal membrane breaks through and releases urine into the amniotic cavity. From this time on, the allantois looks collapsed as if it had expelled a considerable portion of its contents back through the urachus and cloaca and out into the amniotic cavity. And in harmony with this interpretation the amnion in stage 34 appears rather bloated. These changes occur between 12 and 24 hours before birth.

Miscellaneous details. The lateral palatine processes are forming in the mouth on either side of the tongue. The caecum and small intestine still protrude through the umbilicus.

All cranial nerves reach the blastemata of their end organs. The third, fourth, fifth and sixth lumbar, and the first and second sacral nerves develop intercommunications which represent the beginning of the lumbosacral plexus.

There is a well-developed septum spurium in the heart. The conus is divided into pulmonary and aortic halves; and the twisting of the conus has advanced until the pulmonary half is now anterior.

The posterior semicircular canal of the inner ear has been formed. A diverticulum from the nasal epithelium into the septum represents Jacobson's organ. Mihalkovics (1898) commenting on Rose's (1893) description of this organ in the opossum, points out that it is like that of rodents except that it opens into Stenson's duct as in monotremes. Also the fact that its duct arises not from the anterior end of the organ but from the floor behind is a sauropsidan character.

The metanephroi have migrated up to the lumbar region but still consist only of the pelvis and condensed mesenchyme surrounding it.

Hanson ('20) observed the coracoid cartilage in an early stage 33 opossum embryo (H.E.C. 924) extending all the way from the scapula to the sternum without sutures. Later in the same stage (16177) the middle portion of the coracoid disappears and the sternal and scapular ends remain in cartilaginous form. This is the condition when born. In later stages the sternal portion fails to ossify and is resorbed. The scapular portion becomes ossified and attached to the scapula as a typical mammalian coracoid process.

The membrane bones of the skull are layed down in stage 33 but not ossified. All cartilage bones show chondrification except the periotics. Ossification has begun in the clavicle and in the mandible and maxilla. Precartilaginous mesenchyme is recognizable around the trachea.

The first definite anlage of the spleen can be recognized in the thickening of the peritoneal epithelium of the lft side of the dorsal mesogastrium. Cells are proliferating from this point into the mesenchyme below.

The dorsal and ventral pancreatic anlagen unite into a single pancreas. The single duct is that derived from the ventral portion.

XIII. THE THIRTEENTH DAY. Stages 34 and 35

The first half of the thirteenth day

Stage 34. External distinctive features. The abdominal, epigastric and umbilical veins. The diaphragm. The perineum. The oral shield. The palate. The mammary anlagen. Connection between thoracic duct and jugular sacs. Miscellaneous details.

Fig. 53 Photographs of 16179 (stage 34). A, ventral view showing oral shield very clearly. Lateral view showing epitrichial growth over eyes, ears, and sides of mouth.

External distinctive features. Stage 34 is distinguished from stage 33 by the epitrichium, which covers the eyes, ears, and the sides of the mouth; by the oral shield (fig. 53, A); by the claws on the forefoot and the digital ridges on the hindlimb paddle; by the absence of any external intestinal loop; and by a noticeable elongation of the trunk. It is distinguished from stage 35 by the well-developed oral shield; and by the fact that the digital anlagen on the hindlimb are ridges, not buds.

The abdominal, epigastric and umbilical veins. With the collapse of the allantois in stage 34 retrogressive changes become noticable in the umbilical veins, so that this seems to be a good point at which to review their history and comment on their significance.

In stage 24 when angiogenesis begins, one of the first vessels to appear in the body is a vein running along the lateral body wall. This vein, which has been described as the umbilical, is at first the vein of the anterior limb ridge. In later stages it grows caudad through the lateral body wall until in stages 28 and 29 it reaches the level of the developing posterior limb ridge, which it then invades. At this time the postcardinal has also reached this level and the two form numerous anastomoses within the posterior limb ridge. Simultaneously another complication is introduced by the developing allantois, which becomes involved in this umbilical-postcardinal plexus. As the allantois enlarges and projects beyond the body wall in stages 29 and 30, it carries its portion of the plexus with it, and is thus connected with both the umbilical and postcardinal veins.

In stage 30 the rounding up of the body wall carries the umbilical vein further ventrad, and causes its direct connection with posterior limb ridge to become reduced and eventually lost. Its connection with the anterior limb ridge (the radial vein) is preserved for a longer time, but also eventually (stage 31) disappears. The connection with the allantois, however (and thus indirectly with the hindlimb bud through the postcardinal-allantoic plexus), is strengthened, and from this time on the umbilical vein is devoted almost exclusively to the transfer of blood from the allantois to the liver, and is homologous with the umbilical or placental vein of higher mammals.

The connection between the allantois and the postcardinal is retained at first as a plexus of small veins in the middle of the ventral abdominal wall, running caudad and joining the postcardinal at and around the mouth of the external iliac vein. Later (stages 33 and 34) this plexus is reduced to two

veins in the body wall which empty into the external iliacs, and two in the bladder which empty into the internal iliacs or hypogastrics. McClure ('06) saw the two abdominal veins in the body wall of an 8-mm. opossum (my stage 33). These are strictly embryonic, disappearing in stage 35. The two which with their tributaries drain the wall of the bladder into the hypogastric become the vesical veins of adult anatomy.

The developmental history of this abdominal-umbilical series of vessels has an interesting phylogenetic parallel. In some elasmobranchs there is on each side of the body a lateral abdominal (or epigastric) vein which takes blood from both the pelvic and pectoral fins to the duct of Cuvier. This corresponds to the umbilical vein in the opossum embryo of stage 28. In amphibians and reptiles the hindlimb drains into both the abdominal and postcardinal veins, which is the situation in the opossum of stages 28, 29 and 30. In Amphibia and some reptiles the lateral abdominal veins may migrate to the midventral line and fuse into a single anterior abdominal vein which runs from the external iliac up to the liver and through the latter to the sinus venosus (Kingsley, '26, p. 326). In the opossum the umbilical veins thus unite in stage 34. In the birds the same thing occurs, but in addition, the bladder, instead of remaining entirely within the body in embryonic stages, expands through the umbilicus to form an allantois which, being in the path of this abdominal vein, utilizes it and gives it a new significance. This as already described, is precisely the history of the umbilical vein of the opossum during the last $2\frac{1}{2}$ days of development. The paired, lateral, abdominal veins thus become the single, ventral, umbilical.

In the placental mammals this umbilical vein is the vein of the placenta, which is formed from allantoic mesoderm. We can, therefore, trace the placental vein of the highest vertebrates back to the lateral abdominal vein of the sharks. The location and course of this lateral abdominal vein, its connection with both limb buds in the opossum embryo, and with both fins in the shark, suggest that if the paired limbs and paired fins of vertebrates were evolved from a primitive paired finfold, as is usually taught, then this vein was originally the

vein of the finfold. At any rate, it is the original vein of the limbs from whatever source they came.

The diaphragm. The origin of the septum transversum by the lateral and ventral expansion of the sinus venosus, the origin of the pleuropericardial membrane by the medial migration of the ducts of Cuvier; and of the pleuroperitoneal membrane as a ridge which forms at the junction of the pleuropericardial membrane and the lateral body wall, have already been described.

The pleuriperitoneal membrane was described as continuous anteriorly with the anterior tip of the mesonephros, and posteriorly with the coronary appendage of the liver (i.e., dorsal pillar of the diaphragm). Between these two ends the edge of the membrane was in stages 31 to 33 directed toward the interval between the lungs and the mesonephros. Between stages 33 and 34 a considerable elongation of the trunk occurs, which results in carrying the mesonephros caudad away from the lungs. This movement draws the pleuroperitoneal membrane caudad around the posterior tip of the lung. It then fuses with the dorsal mesentery and separates the pleural cavity completely from the peritoneal cavity. Muscle tissue appears in the pleuroperitoneal membranes at this time, and the diaphragm is complete.

The perineum. Although figure 54 is taken from a specimen of stage 35, except for the prominence of the phallus it will equally well represent stage 34. Here it may be seen that the tail gut has completely disappeared and the perineal fold has advanced ventrally until it has divided the cloaca into a cranial urinogenital sinus and a caudal rectum. It is important to notice that the true cloaca has thus been eliminated before birth and the anal and urinogenital apertures are separate. There is a widespread impression, which dates at least from the time of Selenka, that the adult female opossum has a cloaca. This is due to a misleading appearance caused by a common sphincter muscle which develops secondarily around the anal and urinogenital apertures as will be described in the chapter on the pouch young.

The oral shield. Figure 53, A, shows a front view of a curious, cornifield epithelial structure which develops around the oral aperture during the last day of gestation. This apparently useless structure is the most conspicuous and characteristic external feature of stage 34. Selenka (1887, S. 157) first described and figured it, and called it the 'Schnabelschild.' His account is quoted below with the exception of his timing data, which are incorrect.

Fig. 54 Urinogenital reconstruction from 17173 (stage 35), showing well-developed mesonephros, only the beginning of a metanephros, and no müllerian duct.

The most remarkable structure of the epidermis is the oral shield, which is illustrated in Plate XXVII . . . . Some hours before birth it shows signs of degeneration, and in the new-born there is only a trace of it left. The complete shield (fig. 5) surrounds the mouth like a flat collar. Its circumference is raised into six prominent points, the lower ones of which are quite sharp. The whole structure is composed of nothing but cornified, epidermal cells.

When I first observed this remarkable lobate structure I was reminded of the horny bill of the monotreme, Ornithorhyncus, and I believe I make no mistake in recognizing in this

extuberance the vestige of a horny bill which served the ancestors of the opossum as a grasping organ. It can hardly have any function in the opossum, since at the time of birth it has already degenerated, and can not, therefore, participate in tearing the embryonic membranes.

This structure sometimes appears as early as the end of the twelfth day and then persists through the early part of the thirteenth. Similarly I have obtained one batch of stage 33 embryos 11 days and 9 hours post coitum (i.e., before the middle instead of after the middle of the twelfth day). These last four stages are of rather long duration, and, of course, like all other stages, they are not sharply demarcated but grade into one another. I have placed them in the temporal scale where they seem most typical. Some sources for errors of a few hours in the timing of stages are mentioned in the next section in connection with the discussion of the length of gestation.

The palate. Presumably as a result of active opening of the mouth, the tongue drops below the level of the lateral palatine processes which then come together in the midline and fuse below the nasal septum. In their anterior third they also touch the septum and fuse with it. In regard to the opening of the mouth and the relation of the position of the tongue to the lateral palatine processes, it should be mentioned that though the mouth is open to some extent in stage 33 (see frontispiece and fig. 49) the tongue never slips below the palatine processes at that time. The tip of the tongue in the frontispiece is projecting through the incisive foramen, the body of the tongue lies dorsad of the palatine processes, the frenulum of the tongue lies in the narrow slit between the palatine processes. Only the tip of the tongue is seen in this figure. In the posterior part of the angle between the lips the lateral palatine process hides the body of the tongue from view. The oblique line across the mouth is the anterior edge of the palatine process.

In stage 34 though the epitrichium has covered over the sides of the mouth, which gives the impression that the jaws

have been brought together, actually they are farther apart than ever before and the mouth cavity internally is quite large.

Mammary anlagen. The cross-cut surface in figure 54 shows an epidermal thickening between the midline and the base of the hindlimb. Medially the thickening projects deeply into the mesenchyme, and on the surface of the body it lifts up slightly above the surrounding epithelium. This is the anlage of a single mammary gland. There is no continuous mammary rige at first, but these separate anlagen arise in pairs which are arranged into two lines. The most anterior pair appears first, and is situated just craniad of the level of the umbilicus. The more caudal pairs appear in succession forming two lines of separate anlagen which converge toward the phallus. There are usually six such pairs forming a sort of horseshoe, and in addition, a single, unpaired, median nipple at about the level of third nipple from the posterior end in the lateral series. Fairly often there are additional pairs at the anterior ends of the lateral series so that the total may be fifteen or seventeen. In the case of one embryo (17173) I have found the 'median' nipple also paired, making a total of eighteen. In adult females the most anterior pair is sometimes lost, leaving only eleven glands in all. The average number, however, is thirteen. These appear in both male and female embryos, and sex will not be distinguishable in any way until stage 35.

Connection between thoracic duct and jugular sacs. In stage 33 a medial sprout from the jugular lymph sac on either side was described as growing toward the region of the thoracic duct. And it was mentioned that at the same time separate lymphatic anlagen of perivascular origin were forming in the mediastinum in two longitudinal rows which diverged anteriorly from the median, single portion of the thoracic duct. By the coalescing of the perivascular lymphatic anlagen and the medial sprouting of the jugular sac extensions the thoracic duct attains connection with both jugular sacs in stage 34. This makes its anterior end complete, but posteriorly there is no cisterna chyli as yet.

The venules around which the arms of the Y-shaped thoracic duct form are tributaries of the common cardinal and precardinal veins principally, with a few in the more posterior levels from the supracardinals.

The thoracic duct proper, exclusive of the cisterna, is thus seen to be derived from three sources: 1) medial outgrowths from the paired jugular lymph sacs; 2) mesenchymal vacuoles in the mediastinum not associated with venous channels; 3) peri-intimal spaces around degenerating mediastinal venules. The venules referred to are tributaries of the postcardinal, supracardinal, common cardinal, and precardinal veins. The postcardinal and supracardinal contribute the plexus around which the anlage of the single median part of the duct forms. The common cardinal and precardinal veins supply the plexus around which the bilateral arches connecting with the outgrowths from the jugular sacs develop.

In the same way, the external mammary lymphatics, which also arise independently from perivenous spaces, attain communication with the intermediate caudal jugular extension. Further caudad the external mammary lymphatics develop connections with the deep axillary portion of the jugular lymph sacs, and sometimes with the subclavian vein near its origin.

Miscellaneous details. The chorioid plexus is beginning to form in the fourth ventricle. The cerebellar thickening extends across the midline (Larsell, '35, figs. 20 to 37). The pontine flexure is sufficiently far advanced to cause the head to lift away from the chest.

Semilunar valves have formed in the aorta and in the pulmonary trunk. Mesenteric branches of the omphalomesenteric veins have become the principal channels, as the vitelline plexus is showing retrogressive changes. The allantoic vessels are also becoming reduced in caliber.

The lateral semicircular canal of the inner ear is formed. Jacobson's organ elongates.

The tail contains about 22 somites.

Chondrification has at last begun in the dorsal part of the periotic capsule or petrosal bone. Tracheal cartilages are forming.

The second half of the thirteenth day

Stage 35. External distinctive features. The gestation period. Parturition. The migration to the pouch. The negative geotropism. The attachment to the nipple. The respiratory bronchioles. The intranarial epiglottis and the pumping of milk. The 'tubular' muscle fibers. Testis cords and the urinogenital organs. The heart and the cardiac veins. The stomach and the intestines. The sensory organs. The cisterna chyli. The auditory ossicles. Miscellaneous details.

External distinctive features. Stage 35 includes specimens shortly before birth (17173) and shortly after birth (17174 and 17175). All of these specimens are distinguished from stage 34 by the almost complete resorption of the oral shield (fig. 55). Of course, the specimens after birth also show some external changes associated with the new mode of life, as the navel in place of the umbilical cord, and the thicker trunk due to the inflation of the lungs and the distension of the stomach with milk.

The gestation period. Estimates of the duration of gestation, ignoring for the present the uncertainty about the exact time of fertilization, should be based upon the observation of both copulation and parturition in a considerable number of cases. Such information is not easy to obtain in the opossum. The complicating factors are several. Both copulation and parturition occur most often at night. Copulations which take place during daylight hours seem to be most frequently sterile (see chapter I). But whether fertilization occurs or not, a series of changes simulating pregnancy follows each ovulation. There is no visible enlargement of the abdomen even in late pregnancy, for the newborn opossums are so small that even a large litter takes up no more room that a spoonful of baked beans. Any meal will change the shape of the female

more than pregnancy will. The changes in the mammary glands and genital organs during pseudopregnancy are so similar to those of true pregnancy that so far no one has been able to distinguish the two by any means but one—removing one whole uterus and opening it to see whether it contains embryos or not. Selenka, Hartman and I have all used this method. If the technique has been sterile and hemorrhage has been avoided, the incision may then be sutured and the

Fig. 55 Photograph of stage 35 (17175).

remaining uterus left for any desired number of hours or days before being removed in turn. Or it may be allowed to complete its normal gestation. As the embryos in the two uteri are always at the same stage of development, by removing the two at different times one can obtain accurate information about the intervals between the different stages of development. The operation does not retard the development of the embryos in the untouched uterus or interfere with parturition in any way.

Selenka did not have the good fortune to observe parturition, but by removing the two uteri at different times and at known intervals after copulation he obtained accurate information about the timing of twelve of the thirty-five stages I have defined. According to my stage numbers the stages he thus located are 13, 14, 18, 22, 24, 27 (slightly abnormal specimen, but accurately timed), 28, 29, 32, 33, 34 and 35. His timing is accurate only when computed from copulation, which can be done from the data he gives; but the ages as he recorded them (in terms of hours after the beginning of cleavage) are inaccurate on account of the mistake he made about the time of cleavage. His cleavage stages are all degenerating unfertilized ova, as already pointed out by Hartman. The cases in which he described internal structures accurately are noted in the appropriate parts of this text, but in many stages he omitted the internal anatomy.

Though he did not observe parturition, on one occasion he found a litter of young in the pouch 13 days after an observed copulation. And on another occasion he found embryos still in the uterus 12 days and 18 hours after an observed copulation. He concluded that birth must take place about 12 days and 21 or 22 hours after copulation.

Hartman ('28) using the same method was able to assemble a large number of stages, the intervals between which were accurately known. But in only one case did he observe parturition, and in this case, unfortunately, he had not observed copulation. So his estimate of the length of gestation was based upon a known maximum—the age of the youngest litter he found in the pouch—and corroborative evidence derived by totalling the known intervals between a large number of separate stages. In this way he arrived at a figure similar to that suggested by Selenka—i.e., in the neighborhood of 13 days.

I have found a litter in the pouch 12 days and 19 hours after an observed copulation. In conjunction with Selenka's case of unborn embryos at 12 days and 18 hours, this would seem to locate the time of parturition pretty accurately at about 12 days and 18½ hours.

It is probably correct to think of gestation in the opossum as so consistent and precise in duration as to be defined in terms of hours, not days; for when the whole period is so short and development is so rapid, a difference of a few hours makes a considerable difference in the stage of development, and it is not likely that there can be any great irregularity about the stage of development at birth. On the other hand, all of the above estimates suffer from one defect. Copulation in the opossum is not a momentary affair. It sometimes lasts for 5 hours. When, then, is one to say copulation occurred? My own notes always refer to the time when intromission was first observed. If the time of fertilization could be determined accurately, that would be the proper starting point, but as long as our information about that is only approximate, our knowledge of the length of gestation must be only approximate.

With the above reservation, then, all the evidence seems to point to a rather sharply defined gestation period of about $12\frac{3}{4}$ days.

Parturition. The mother sits on her haunches and licks the vulva, the abdomen, and the inside of the pouch. Contractions of the uterus probably force the young into the vaginal cul-de-sac. The chorions, which are all fused into a single mass and intimately associated with the mucosa of the uterus by complex folds, are left in the uterus. The amnion and fragments of the allantois go with the young into the vaginal cul-de-sac. Contractions of the maternal abdominal wall then cause the lateral vaginal canals, which are bent upon themselves, to become completely occluded, and the increased intra-abdominal pressure can be relieved at only one point—the pelvic canal, which leads to the outside. The posterior wall of the vaginal cul-de-sac is thin and lies in contact with the anterior wall of the urogenital sinus, the two together forming a delicate membrane across the pelvic canal (fig. 5). This membrane ruptures under the increased pressure from the inside, and an artificial, but direct, passage—the pseudo-vaginal canal—is formed. The young pass through this into the urinogenital sinus, and thence to the outside.

The amnion and the allantois are exceedingly thin and delicate. If the surrounding fluid is removed, the allantois will usually break under the weight of its own contents. The amnion is so close-fitting and thin that a single stroke of the foreleg will rip it open. These two membranes are correspondingly torn to shreds very early in the course of parturition, and are usually left in fragments in the vaginal cul-de-sac and urinogenital sinus, though some fragments may sometimes be still adhering to the foetus when he emerges. No such fragments are ever found in the lateral vaginal canals, for the foetus does not pass out that way.

On emerging from the vulva the newborn is thus either entirely free of its membranes, or attended only by fragments of the amnion and allantois. The statement sometimes made that no navel is visible at this time is due either to special use of the word 'navel' or to failure to look in the right place. There is no depression, but the interrupted epidermis and the torn end of the urachus are always distinctly visible a short distance above the tip of the phallus (fig. 56). In this position the navel tends to be concealed by the hind legs and the tail (fig. 55), and careful inspection under the dissecting microscope is necessary to identify it.

The migration to the pouch. The method by which the newborn marsupial gets from the urinogenital sinus to the pouch has long been a subject of dispute, and though exact information with reference to the opossum has been available for some time (Hartman, '20), a large number of naturalists remain somehow unconvinced.

The original theory, and one which is still widespread today, is that the mother picks up the newborn young with her lips, places it in the pouch, and holds it there until it is attached to the nipple.

Middleton Michel published in 1847 what purported to be a first-hand account of parturition in the Virginiana opossum. I do not mean to reflect upon the good faith of Doctor Michel's report, but in the light of subsequent observations by several others, I think it possible that he was misled by appearances

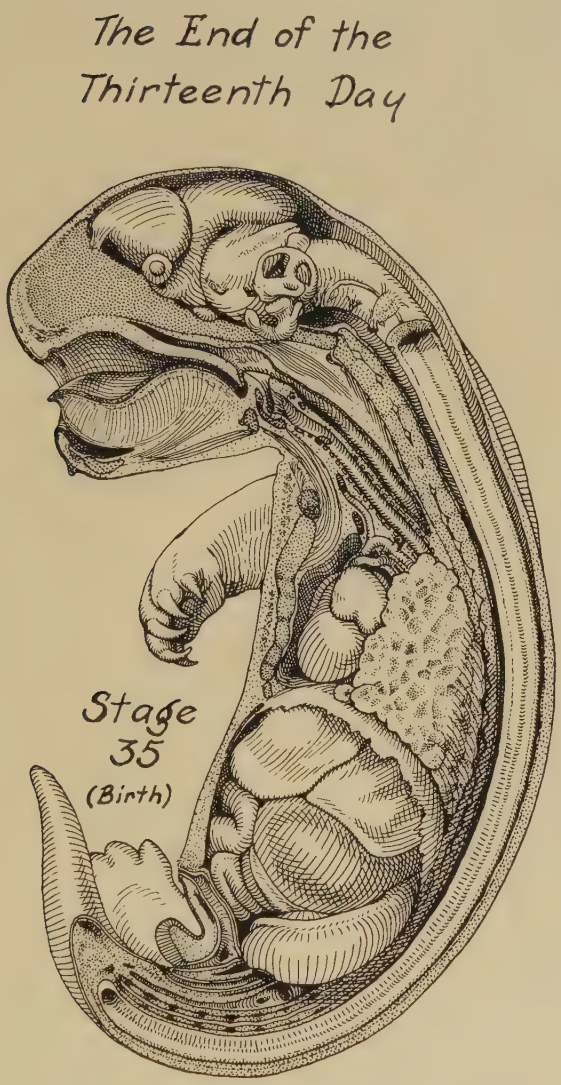

Fig. 56 Drawing from dissection of specimen 17184 (stage 35). To obtain this view of the membranous labyrinth the periotic capsule was removed and cleared in aniline oil.

into thinking that he had observed parturition when he really had not.

On January 28, 1847, Michel observed the copulation of two opossums, and 14 days and 17 hours later he observed what he described as follows:

> The pregnant female was found standing on her hind legs; her body was much bent, and propped up against the corner of the cage; her muzzle in immediate contact with the cloacal opening, which was red, tumified and distended; a young appeared at the opening, and was conveyed by the mother's mouth to the pouch, or perhaps was rather licked in, as her tongue seemed busily employed within, around and about the pouch.

The details which make this account questionable are several. In the first place, the period of 14 days and 17 hours is about 2 full days too long for normal gestation. As mentioned in a previous section, it is unlikely that the period varies more than a few hours. A variation of 2 days is certainly abnormal, if possible at all.

In the second place, the external genital region is not 'tumified and distended' at the time of parturition; though to one not thoroughly familiar with its appearance, it might easily appear to be so at any time, for it always has a 'swollen' appearance. That he was not very familiar with its appearance is definitely shown by his reference to the 'cloacal opening.' A closer observation would have shown him that there is no cloaca at all in the opossum. The urinogenital orifice and the anus open separately to the outside (fig. 5). Finally, the wording of his last sentence clearly expresses uncertainty about what he saw. The female was licking the perineal region, the lower abdomen, and the inside of the pouch. The young that he thought he saw at the opening may very reasonably have been the tip of the mother's tongue, for I have often seen it when it could easily be thus misinterpreted. It seems rather likely that the young he found in the pouch had been born about 2 days previously, and all he observed was the mother grooming herself.

Twenty years later another observer (E. S. Hill, 1867) thought that he had seen a similar thing in the kangaroo. While concealed behind a bush watching some wild kangaroos he thought he saw a female pick up 'a stone' or something in her lips. He then saw her put her head into her pouch; and later when he shot her, he found a young foetus inside. The female very commonly licks out the inside of her pouch. He did not recognize the foetus in her mouth; in fact, did not even think of a foetus until he found one in the pouch. Then, of course, it was very easy to interpret what he had seen as he did; especially, as one must admit that a very young marsupial looks altogether incapable of getting into the pouch by its own efforts.

These two observations form the basis for the theory that the mother transfers the young from the vulva to the pouch by means of her lips. One of the very serious objections to the theory is the fact that in some marsupials (e.g., the bandicoots, Peramelidae, and the marsupial mole, Notoryctes) the opening of the pouch is directed backward, and it is unlikely that the mother can insert her snout into the pouch at all.

The next important evidence was published in an Australian newspaper in 1913, but was not made generally available until Hartman republished it in his 1920 paper. Mr. A. Goerling had been attracted by the curious behavior of an adult female kangaroo (Macropus rufus) which he had in captivity. She had refused food, and he had observed blood marks in the cage. She was sitting with her "tail passed forward through the legs," and "was busy licking and cleaning herself." Goerling says,

Presently she lifted her head, when I was astonished to see a young kangaroo clinging to the long fur about four inches below the opening of the pouch. It moved about slowly, very slowly, through the fur upwards, using the arms in its progress, and continually moving the head from side to side, thus assisting the upward movement. Nearly 30 minutes were required by the little wanderer to reach the top of the pouch, the last end in a semicircle. During the whole

of this time the mother paid no attention to her offspring, offering no assistance, and leaving it entirely to its own exertions.

This was the first time anyone had seen a very young marsupial crawl up to the pouch by its own efforts, but as the author had not actually seen the young emerge from the urinogenital sinus, he could not prove that it was actually newborn. The next question to be settled was: What is the earliest age at which the marsupial young is capable of crawling by its own efforts?

Hartman ('17) made some experiments which answered this question, but which he did not publish until 1920. He removed embryos near term from the uterus, freed them from their envelopes, and allowed them to crawl about over the mother, "which they did for at least fifteen minutes." This definitely established the fact that they are capable of this complicated feat even before birth.

The final link in the evidence was supplied on February 6, 1920, when Doctor Hartman and Mrs. Hartman both witnessed the whole migration from the first appearance of the foetus at the vulva until its attachment to the nipple in the pouch. "Unerringly the embryo traveled by its own efforts; without any assistance on the mother's part, other than to free it of liquid on its first emergence into the world" (Hartman, '20, p. 5).

I have given this rather lengthy summary of the evidence because it does not seem to have found its way into the textbooks of zoology, and the impression that the mother places the young in the pouch is still almost universal. This must be due partly to the extremely immature appearance of the newborn and the consequent difficulty of believing it possible that such a foetus could accomplish so difficult a feat. It may also be due in part to unsuccessful attempts by others to demonstrate this ability in young removed from the pouch for experimental purposes.

I have performed such experiments many times, and for a while without success. My first success came on an occasion

when I had called in two witnesses. Thirteen days and 2 hours after an observed copulation a young opossum had been found in the pouch (its actual birth not having been observed). I removed the foetus from the nipple and placed it on the mother's abdomen. The hair was dry and the foetus stuck to it so that although he struggled vigorously he was unable to make progress in any direction. Wetting my finger I moistened all the mother's hair in that region with saliva, and also moistened the foetus' skin. The latter's movements quickly became effective. In less than 3 minutes it had clambered up the inclined plane of its mother's abdomen and disappeared into the pouch. Three minutes later I open the pouch and found it safely attached to another nipple—not the one it had first appropriated.

The significance of this experiment seems to be that though the mother does not pick up the young, her licking the hair of the abdomen seems to be an essential service.

The negative geotropism. Hartman ('20, pp. 255 and 256) pointed out that, "If the skin be tilted, the embryos can be made to travel upward and even away from the pouch, for they are negatively geotropic." Langworthy ('28, p. 214) noted that the vestibular mechanism does not begin to function until about 41 days after birth. So it is evident that if the young does orient itself away from gravity, it does so without the assistance of any vestibular mechanism.

Both of these observations have been confirmed and an explanation suggested as follows (Larsell, McCrady and Zimmermann, '35, pp. 106 and 107):

> The effect of gravity on the position of the body of the pouch young, before vestibular reflexes appear, is obvious when the young are placed upon a rough towel or a hairy arm. The forefeet are well enough developed at birth to make possible grasping of hairy or similarly rough surfaces, but the hind feet do not have this capacity. When the surface upon which the young is placed is tilted at the proper angle, the body, held in position only by the grasping action of the forefeet, swings in obedience to the pull of gravity, the outspread hindlegs serving only to brace it and prevent it from rolling. In this manner the head is always directed upward.

The young thus orients itself away from gravity without the assistance of a righting reflex or of any consciousness of 'up or down.' This is a true tropism, or simple, automatic orientation, conditioned solely by the structure of the body at the time—the functional forelegs and the non-functional hind legs.

The mother sits in such a position that the path away from gravity leads to the pouch. And as a result the young wanders blindly into the place of safety and food. Probably many of them miss it, for the young found in the pouch are nearly always fewer than the embryos found in the uteri shortly before birth.

The crawling movements have been studied in some detail. The youngest foetuses which I have tested for reflexes were 11 days and 9 hours after copulation (34 hours before their litter mates from the other uterus were found in the pouch). These stage 33 foetuses were placed in mammalian Ringer's solution. Their vascular system was intact—no embryonic or extra-embryonic vessels either ligated or cut. Their hearts could be seen beating. No foetal respiratory movements were observed. Hairs and fine gut sutures (medium dermis suture —size 000) were used as probes. No reflexes could be evoked by tactile stimuli on any part of the body. Unfortunately, only superficial stimulation was tried, so I am unable to say whether the muscles can respond to deep, direct stimulation, but this is not likely, as not even fixation in Bouin's fluid will cause any muscular contractions at this time. The most serious objection to this experiment is that the Ringer's solution was not artificially heated. The temperature of the room and of the solution at the time was probably about 85°F.

Late stage 34 foetuses, on the other hand, if removed from the uterus and freed from their membranes, execute perfect crawling movements like those of the newborn to be described next. So the development of this complicated behavior is very rapid—the non-motile to the crawling stages in 24 hours or less.

The crawling foetuses of stages 34 and 35 are so ceaselessly active that I have not so far devised a successful method of testing them for reflexes to tactile stimulation. But the crawling movements themselves are interesting. These begin with the lateral flexion of the head and neck followed closely by the forward movement of the ipsilateral foreleg. The head then starts a flexion toward the opposite side while the claws of the forefoot close in a clasping movement and the foreleg is moved backward in an effective, propulsion stroke. The caudal end of the body is then flexed as a whole toward the side on which the effective stroke of the forelimb is being executed. This constitutes a complete cycle. A similar cycle is initiated on the contralateral side when the head moves in that direction. The whole cycle seems to represent a wave of contraction which starts at the anterior end of the body on one side and travels posteriorly, the similar wave of the opposite side starting before the first wave has progressed beyond the anterior limb. This is a typical Coghill ('29) S-reaction except for the precocious individuation in the forelimb.

The attachment to the nipple. When the young enters the pouch he finds himself in a maze of tangled, curly hairs among which he must locate one of thirteen, tiny mammary nipples—about the size of the head of a common straight pin. The sensory equipment which he has to assist him in this search, is very scanty indeed, as will be described below. And perhaps the most amazing thing that he does is to succeed in this search; but once in the pouch, he is almost certain to do so. I have watched them do it many times, and I do not yet know what clues they use—certainly not sight, possibly smell, possibly cutaneous sensations of warmth, moisture, and touch. The head is moved in wide excursions to right and left, and when the snout finally touches the soft, moist, and warm nipple, the wandering stops and the young promptly sucks the nipple into its mouth.

The sealing of the lateral portions of the lips by means of epitrichial cells in stage 34 leaves the mouth large inside,

but with only a small aperture to the outside. When the soft nipple is sucked through this narrow opening it swells up on the inside and 'buttons' the young to the mother. For several days this is a weak attachment, but the more the nipple is sucked upon, the more it enlarges at its tip, and the more secure the attachment becomes. After about the fourth day in the pouch the young cannot be removed without considerable effort and danger of tearing the corners of the mouth. This is not due to any organic union between maternal and foetal tissues, as has been popularly supposed, but simply to the fact that the oral aperture is not large enough to permit the

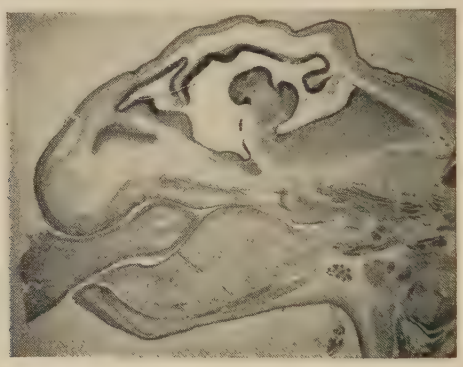

Fig. 57 Photograph of section from an 8-day post partum opossum with the mammary nipple still in its mouth. No fusion of maternal and embryonic tissue occurs.

passage of the bulbous end of the nipple. Figure 57 shows a sagittal section through the head of a young mammary foetus with the nipple in the mouth.

There is also a traditional belief that the young swallows the nipple all the way down to the stomach. This is based upon the observation that after about 6 or 7 weeks of nursing, the nipple stretches to some 1½ inches in length. But only the tip of this is ever in the young's mouth. The epiglottis is so constructed (v.i.) that the nipple cannot pass it. The long nipple allows the advanced young to nurse from outside the pouch when they have become too large for the pouch to contain them all at once.

The respiratory bronchioles. Selenka (1887), who was the first to describe the lung of the newborn opossum, said:

The construction of the lung is precocious in the sense that alveoli capable of respiratory function are developed in an extraordinarily short time; but it is retarded in the sense that the multiplication of the alveoli is deferred to a later time. Probably also hereditary phenomena are at work in this strange developmental history of the opossum lung, for the lung of the new-born has exactly the appearance of a reptilian lung; and since the simple structure of the latter is manifestly adequate for the marsupial young, it can be retained during the early part of life. Not so with the Placentalia, among which the embryo is retained much longer in the uterus. Indeed, even with them the first anlage of the lung is much like that of the reptile and the opossum, but these simple alveoli do not function any longer as respiratory organs, having now become the building loci for the definitive infundibula and alveoli.

Bremer ('04) confirmed Selenka's description in general, but suggested that it is incorrect to call these first functional air chambers 'alveoli.' Histologically, they are really peculiar, modified bronchioles, which function as respiratory chambers for awhile, and then revert to typical bronchioles after the true infundibula and alveoli have grown out from them. The difference between these first 'alveoli' and the true alveoli seems to have been recognized by Selenka in his last line where he distinguishes between 'die einfachen Alveolen' and 'die definitiven Alveolen,' but Bremer's terminology is probably better. A rather more fundamental point was made by Bremer in correcting Selenka's impression that the true alveoli are formed by the ingrowth of partitions which subdivide the primitive respiratory chambers. Bremer pointed out that the infundibula and true alveoli arise as diverticula from the respiratory bronchioles.

One interesting point about these first respiratory chambers is that they are lined with an easily demonstrable respiratory epithelium. The illustration of this epithelium shown in figure 58 is taken from Bremer. The true alveoli do not appear until quite late. Bremer found them in a 14-cm. opossum.

On the average such an animal would be between 70 and 80 days of age. The latter figure represents the usual time of weaning. I have found that the early pouch young cannot control their own body temperature. They are 'incubated' within the marsupium. The control of body temperature seems to begin between the sixtieth and seventieth days. One cannot help but wonder whether the reptilian lung and the poikilothermal condition are correlated. As long as he does not have to maintain a high rate of metabolism to provide his

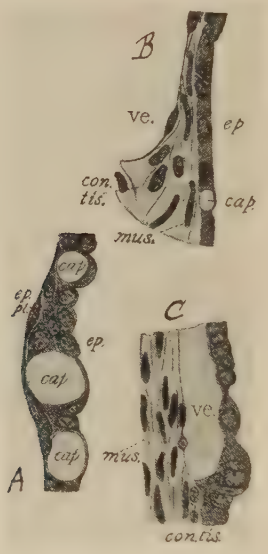

Fig. 58 Functional respiratory epithelium (from Bremer, '04). A and B from a 12.5-mm. opossum. C from a lizard.

own source of heat, the relatively inefficient oxidation accomplished through a visible epithelium may be as adequate for a mammal as it is well known to be for reptiles and amphibians. In the adult opossum, which does control his own body temperature, an epithelial lining is no more demonstrable than it is in any other adult mammal. A careful investigation of the temporal relation between the change in metabolism and the change in alveolar lining should be made (see remarks in the Alveolar Epithelium Conference reported by Macklin, '36).

A few words about the gross anatomy of the lungs are in order. The left lung in the possum is composed of only one lobe, instead of two (fig. 56). The right lung, on the other hand, has in addition to the usual three, an accessory or infracardiac lobe the bronchus of which arises near the hilum of the lung on a level with the first hyparterial bronchus, and runs medially and caudally beneath the heart. Jazuta ('32) observed that the right and left pleural cavities in the opossum communicate with each other in the dorsomedial line. This interpleural opening has been found in Didelphys, Perameles, and various birds. This fact and the fact already referred to, that the functional lung of the opossum at birth is composed only of bronchi and bronchioles without alveoli, Jazuta regards as strong evidence that the Marsupials represent a connecting link between the Eutheria and the Sauropsida.

I have not been able to find in any of my specimens, either before or after birth, a left eparterial bronchus at the level of or anterior to the left hyparterial. Bremer ('04) described such an eparterial bronchus in five newborn opossums, but mentioned that it is always "smaller and slightly lower placed, and the chambers supplied by it *do not form the apex of the lung.*" As in all my specimens the only such bronchus I can find is well below the left hyparterial, I am forced to suppose that what he saw was either an abnormality, or, more likely, the first left dorsal bud, which occurs also in man and in mammals generally, and is certainly of no phylogenetic significance. There are many mammals, both high and low in the phylogenetic series, in which a true left eparterial bronchus has been described—Bradypus, Phoca, Equus, Auchenia, Elephas, Phocaena, Delphinus, and Cebus (to take a list from Goodrich, '30, p. 610)—and at best their phylogenetic significance is very uncertain.

The intranarial epiglottis and the pumping of milk. As the term implies, the epiglottis in the opossum projects into the posterior nares or choanae (fig. 56). It is rather tubular in form, and in this position fluid can pass around it on both

sides, without necessarily interrupting breathing, and without danger of choking. Incidentally, this condition persists in the adult.

Such an an arrangement is found also in all the Cetaceae and in scattered members of all the other mammalian orders. In the case of the marsupial mammary foetus it lends credibility to the theory first proposed by Seiler (1828) that the young does not suck, but has its milk pumped into it by a muscle in the maternal marsupium which is homologous to the cremaster of the male and which he called the iliomarsupialis or compressor mammae. This so well developed in some forms (e.g., Thalacynus—see Cunningham, 1882, pl. 4) as to provide an obvious mechanism for the purpose.

On the other hand, in the opossum there seems to be no mechanism for the pumping of milk, and the newborn foetus can be seen and heard to suck. Indeed, it is only by suction that it draws the nipple through the narrow, external aperture of its mouth. It seems certain that not only the opossum but all newborn marsupials can suck. The pumping of milk by the mother is not universal, and is only a supplemental mechanism if it occurs.

The 'tubular' muscle fibers. The functional muscles in the newborn opossum are histologically unique. Selenka has illustrated them beautifully in longitudinal and cross-sectional views (1887, pl. XXIX, figs. 6 and 7). The fibers are striated, but the nuclei, instead of being disposed peripherally, are aligned in a single axial row. All the striated fibrillae are confined to the periphery. The cytoplasm between the nuclei is clear and undifferentiated.

I know of only one other instance in which the nucleus of a striated muscle cell is located in a non-fibrillar central core. This is in the heart muscle of the gasteropod mollusc, Sycotypus canaliculus (Dahlgren and Kepner, '08, fig. 91), but in this case, as in heart muscle generally, there is only one nucleus in each cell; whereas, in the opossum's tubular fibers there are numerous nuclei in tandem along the non-fibrillar core.

This curious arrangement, however, is only transitional in the opossum, for by the time the pouch young is 2 weeks old all of the nuclei have migrated to the periphery, and the whole fiber is quite like that of any other mammalian striated muscle.

Testis cords and the urinogenital organs. Though sex cannot be distinguished externally until some 12 to 14 days after birth, the first histological differentiation can be recognized in stage 35. The female gonad at this time still apears indifferent, but the male gonad develops clearly defined testis cords containing the primordial germ cells.

At this time there is still no sign of a müllerian duct (figs. 54 and 59). The ureters as they enter the bladder are medial to the wolffian ducts. Tubule anlagen are just beginning to form in the metanephroi. The mesonephroi are highly developed and functional. These primitive kidneys remain functional for about a week after birth in the opossum.

The heart and the cardiac veins. A short resumé of previous stages will help to clarify the following description. During the concrescence of the heart tubes the primary head vein and the umbilical vein on each side fuse together and then develop a communication with the vitelline vein on each side to form a sinus venosus (stage 25). At this time the paired heart tubes have fused only in the bulbar region. In stage 26 the ventricles fuse, and in stage 27 the atria fuse and the two venous sinuses open into the single atrium separately on either side of the foregut. At stage 30 the two sinsuses have come together so that there is only one valve into the single atrium, and the septum primum now begins to form just to the left of this valve, so that when the single atrium becomes divided into two again, this valve empties into the right one of the two new atria.

The twisting of the heart has progressed so far at this time that the left precava is pulled up tight against the atrium of the left side, passes beneath the pulmonary vein, and at the back of the heart lies in the crease between the atrium and the ventricle. Here in stage 35 it receives one or more small tributary veins from the wall of the left ventricle before it

empties into the sinus venosus. This condition persists in the adult, for the two precavae do not develop any cross anastomosis as in Eutheria but remain permanently independent of each other. The left precava thus serves to drain

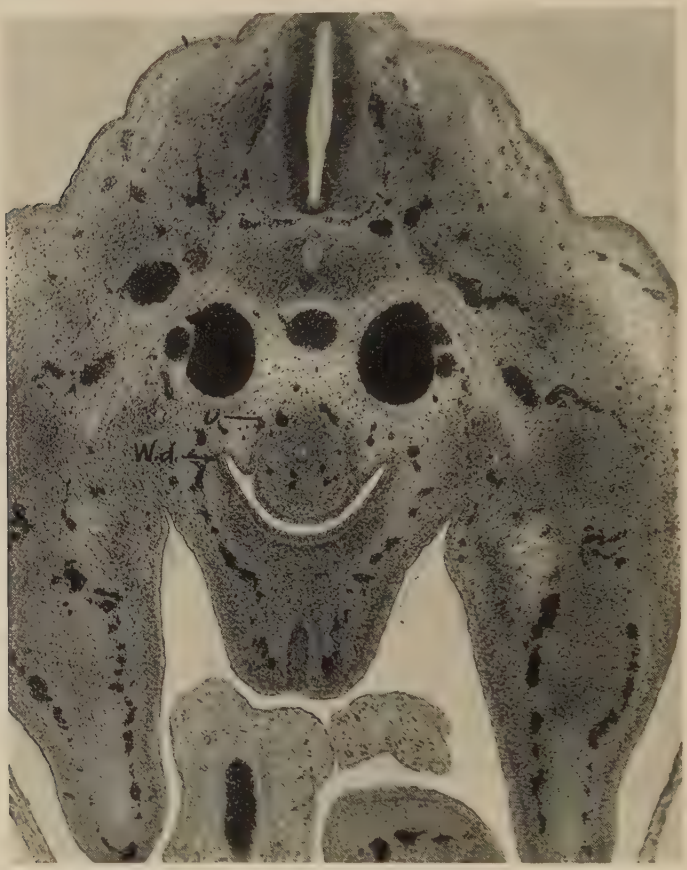

Fig. 59 Photograph by Dr. A. A. Zimmermann of a section through the cloaca in specimen 16175 showing entry of ureters into clocal horns medial to wolffian ducts. No müllerian ducts are present.

the blood from the left wall of the heart, but at the same time retains its original function of draining the left side of the head and neck. It is thus both coronary sinus and precava.

The veins just described as emptying into the precava are the left cardiac veins, but in the opossum the largest cardiac vein, the vena cordis magna, has been shown by McClure ('03) to run in the ventral interventricular furrow until it reaches the auriculoventricular groove, then to pass dorsad between the root of the pulmonary artery and the left auricle and empty into the right atrium as in birds. This is also characteristic of Thalacynus as Cunningham (1882) has shown. Such a condition is never found in placental mammals.

The stomach and the intestines. Heuser ('21) studied the digestive system in stages 34 and 35. He found that the stomach becomes enormously larger immediately after birth due to distension with milk (fig. 60), and that its gross anatomy is like that already described for the adult opossum by Bensley ('02). "Glands however," he says, "are nowhere present, nor can any of the elements be regarded as cells associated with the formation of acid as found in the functioning stomach of higher mammals." The stomach, therefore, is a reservoir at this time, and does not otherwise participate in the digestive process.

The same author observed that:

Villi are present in all parts of the small intestine, although they are older and more advanced in the duodenum . . . . In many places between the villi there are patches of epithelial cells which are smaller and more darkly stained than those covering the villi . . . . The large intestine had changed to a much smaller degree relatively in the transformation from the late embryo to the pouch young. Although the external caliber of the colon has increased considerably in the interval of growth, there is but little advance in the epithelial or mesoblastic differentiation . . . . The relatively slight dilation of the colon and the histological findings indicate that the residue of the foodstuffs reaching the colon in the opossum pouch young is relatively small; practically all of the milk must be disposed of in the upper portions of the alimentary canal.

The sensory organs. The eye of the opossum at birth is in a very embryonic condition. The lens vesicle is in process of developing a fiber nucleus; that is to say, the cells of the medial

wall have elongated, but have not yet obliterated the cavity of the lens. These primitive lens fibers are still nucleate. The pars optica of the retina shows only the cellular and fibrous layers, no ganglionic or rod and cone layers having

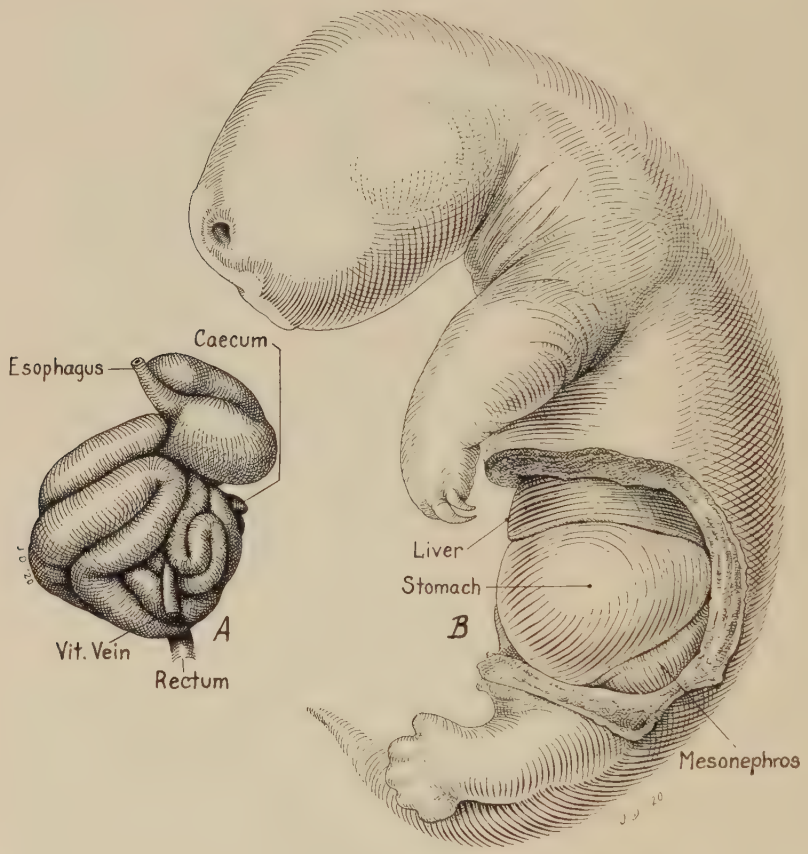

Fig. 60 Dissection of abdominal viscera of embryo and early pouch young (from Heuser, '21). A, stage 34. B, stage 35.

yet differentiated. Pigmentation of the outer layer has just begun. The cornea has not cleared. Mesenchyme which will later give rise to the vitreous body is beginning to filter into the optic cup, through which a single vascular loop runs. This stage is similar to that of a 12.5-mm. human foetus except

that in the opossum the eyelids have become fused together and covered over by the epitricium. Externally there is no indication of the eye, except that in the living specimens the pigment of the retina can be faintly seen through the epitricium.

I have removed the lens from a newborn opossum, and found that 15 days later (17183) there was no regeneration, and in the absence of the lens the cornea had not cleared and the retina had atrophied to a considerable extent. The control eye in the same individual had advanced to a stage similar to that of a 3-month human foetus.

Like the eye, the ear at birth is completely non-functional (Larsell, McCrady and Zimmermann, '35). The cochlear duct (fig. 56) has grown out to about one-half turn. It contains no organ of Corti, but the epithelium of the tympanic wall is slightly thicker than that of the vestibular wall. There are no hair cells, tunnel of Corti, tectorial membrane, scala tympani, or scala vestibuli. The external auditory meatus is completely plugged with epitrichial cells, and the latter also overlie the developing pinna.

In the vestibular portion of the inner ear the ampullae of the semicircular canals are recognizably indicated, and the utricular and saccular anlagen, though not yet separated from each other, are identifiable. The only indication of the cristae and maculae is the slightly thicker epithelium at the appropriate points. There are still no signs of cupulae, otolithic membranes, or perilymphatic space. No nerve endings are present in either the acoustic or vestibular part of the inner ear.

The olfactory organs, on the other hand, may possibly be in a functional condition. The conchae are not well developed (about like those in a 3-month human foetus), but the olfactory epithelium is ciliated, and nerve fibers from this epithelium enter the olfactory lobe of the forebrain in large numbers. Selenka, also judging from histological appearances, considered this organ functional.

There are no papillae or taste buds in the tongue.

Selenka (1887, p. 160) claimed that no organs of touch are present, but perhaps this question has not been approached with adequate techniques. It is hard to imagine that the complicated crawling movements with their necessary component —the clasping of the mother's hair by means of the claws and toes of the forefeet—can be accomplished without some sort of exteroceptive apparatus in the skin.

The cisterna chyli. In the opossum the cisterna chyli is not developmentally a dilatation of the thoracic duct. It is derived principally from a mesenteric lymph sac which develops in stage 35 and which is homologous with the mesenteric lymph sac of rabbit, pig and cat embryos. This secondarily establishes communication with a peri-aortic lymphatic plexus which leads into the caudal end of the thoracic duct. The peri-aortic lymphatic plexus later enlarges and forms the cranial portion of the cisterna, which is permanently rather plexiform.

In addition to the rather poorly developed connections of the mesenteric sac with the peri-aortic plexus in stage 35 there is a well-developed connection with the peri-oesophageal lymphatics. This curious arrangement is later abandoned, only the peri-aortic connections being preserved. Doctor Zimmermann has suggested that the temporary connection with the peri-oesophageal lymphatics is associated with the precocious development and functioning of the digestive tract.

Among other minor changes in the lymphatic system at about the time of birth, the jugular lymph sacs attain their maximal size, and the axillary portions establish communications with the deep lymphatics of the forelimb. The thoracic duct enlarges markedly in caliber, and caudally it attains communication with the mesenteric lymph sac as described above.

No lymph nodes have been developed in any part of the body at birth.

The auditory ossicles. In examining the earliest stages in the development of the ossicles in the opossum I have been unable to find any clear connection between the stapes and

the hyoid arch at any time. As such a connection has been described in a great many mammals this matter needs further study, and I have referred the problem as well as the detailed study of the development of all the ossicles to Mr. J. A. McClain who is now working in this laboratory. Accordingly, I shall content myself in this paper with a brief comment on only two stages.

Figure 61 shows the condition of the ossicles at birth. This is a familiar arrangement and needs very little comment. Meckel's cartilage extends from its articulation with the incus all the way forward through the core of the mandible. The

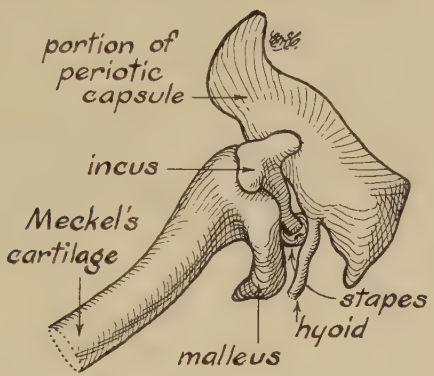

Fig. 61 Reconstruction of cartilaginous auditory ossicles at time of birth (17175). Lateral view.

stapes is penetrated by the stapedial artery, a branch of the internal carotid. The hyoid cartilage is continuous with the periotic capsule and makes no contact with the stapes, which is medial and slightly anterior to it.

Figure 62 shows a much later stage near the time of the beginning of auditory function. This specimen was 49 days of age (i.e., post partum), and was tested for auditory reflexes before he was killed. No reflexes were observed, though in the light of later studies (McCrady, Wever and Bray, not yet published) it seems likely that the first electrical responses of the cochlea could be detected at this time.

The principal point to be noticed here is the extraordinary anterior process of the malleus. This process extends in a semicircle halfway around the tympanic annulus, and it persists in the adult. It is derived, not from Meckel's cartilage, which is also shown in the figure, but from a membrane bone, presumably the prearticular, which partly encloses the posterior end of Meckel's cartilage just as the dentary encloses the anterior end. The portion of Meckel's cartilage lying in the groove of the prearticular degenerates without ossification. All of the manubrium and possibly also the head and neck of the malleus, however, are formed from the extreme tip of Meckel's cartilage. The foramen shown in the anterior process admits the passage of the chorda tympani nerve.

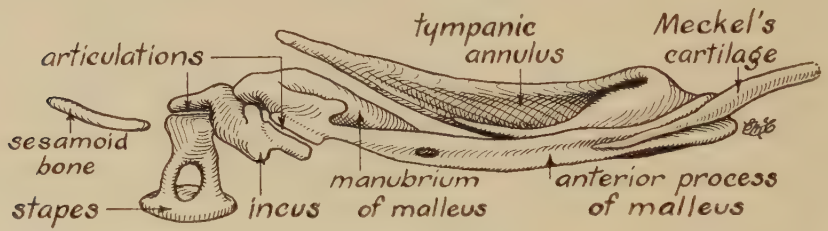

Fig. 62 Reconstruction of ossicles and tympanic annulus 49 days after birth showing the extraordinary anterior process of the malleus. Dorsal view.

My tentative view of the homologies of these structures is: The original mandibular arch becomes subdivided into an upper palatoquadrate and a lower Meckel's cartilage. The posterior end of the palatoquadrate ossifies to form the quadrate of reptiles, which becomes the incus of mammals. The posterior end of Meckel's cartilage ossifies into the articulare of the reptiles, which becomes the manubrium, and possibly the head and neck, of the malleus of mammals. The anterior process of the malleus, which is exceptionally large in the opossum, is formed from a membrane bone, the prearticular of reptiles. Another membrane bone, the angulare of reptiles, becomes the tympanic annulus of mammals.

This accounts for all but the stapes, the only one of the ossicles about which there has been general agreement in the

past. Mr. McClain's more detailed study may yet disclose the connection between the hyoid arch and the anlage of the stapes which I have been unable to find in the opossum. But even if it does not, this connection has been found in so many other mammals that the weight of the evidence will still favor the homology of the stapes with part of the hyoid arch, and with the medial end of the columella of lower vertebrates.

Miscellaneous details. The nasal septum fuses completely with the palate. Stenson's canals remain open. Labiodental ledges are forming in upper and lower jaws. The naso-lachrymal cord is still solid and still does not reach the nasal epithelium. The vomer is represented by two cartilages as in reptiles, rather than by one as in mammals. In the adult opossum, however, there is a single, median vomer, as in other mammals. This is strong evidence of the homology of the reptilian and mammalian vomers, usually considered a moot point.

The interatrial septum still shows a few very small perforations. The ductus Bottali is constricting, but still patent. The interventricular septum is incomplete in the bulbar region. The twisting of the bulbus is complete, so that the pulmonary trunk, which was originally caudal to the aorta, has passed through three-quarters of a turn and now lies to the right of the aorta.

The tail has about 24 somites, which makes the total number of segments formed 52. This is the final number.

The medulla of the adrenal gland is in process of formation from chromaffin cells.

The splenic epithelium separates from the underlying mesenchyme, but there are no splenic sinuses and practically no blood vessels. This situation is similar to that of a 15-mm. pig.

There is no corpus callosum between the cerebral hemispheres, and none ever develops in the opossum. Johnston ('13), however, by means of degeneration experiments has shown that in the adult opossum certain fibers in the dorsal commissure serve the same purpose and probably represent the phylogenetic anlage of the corpus callosum.

The erythrocytes at birth and for some days afterward are oval and nucleate like those of lower vertebrates.

XIV. APPENDIX

Postnatal development. Some experimental techniques. The evolution of the mammals.

Postnatal development. As its title indicates, the proper province of this book does not extend beyond the time when the newborn young reaches the pouch and becomes attached to a mammary nipple. But though embryology proper may be said to end at that time, so much of development remains to be accomplished that a few words about the pouch young seem necessary before closing.

The earliest pouch young (i.e., the newborn opossum described in the preceding chapter) averages about 11 mm. in crown-rump length, though many individuals may be as small as 10 mm. C.R.L. It has a fairly well-developed circulatory system; lungs which are functional, though unfinished; a digestive system which is also functional, but unfinished; a muscular system which is partly functional, but unfinished; a skeletal system which is almost entirely in the cartilaginous and membranous stages; a central nervous system which has virtually no cerebellum (Larsell, '35), but is well developed in the hind-brain and cervical cord; a functional mesonephros and a non-functional metanephros; only the earliest stage of histological sex differentiation in the gonads; non-functional eye and ear, and only non-functional endocrine gland anlagen.

At this stage of development the young opossum becomes so securely attached to its mother's mammary nipple that it cannot detach itself for some 50 days. And during this interval it brings the development of all its organs to a condition roughly approximating that of most newborn placental mammals. A few details of the changes during this 50-day period will be mentioned.

About 1 week after birth the metanephros begins to function and the mesonephros shows retrogressive changes. At about the same time the müllerian duct anlage appears. It arises as an invagination from the coelomic epithelium into the ventral border of the extreme anterior end of the mesonephros. A very early stage of the diverticulum is found in a 17-mm. male specimen lent to me by Dr. C. F. W. McClure of Princeton University. The development of this duct and the part it plays in the formation of the female genital tract have been studied by Dr. J. S. Baxter ('35). The following details are taken from his paper.

The müllerian duct tunnnels into the mesonephros and grows caudad as a solid cord which follows the course of the wolffian duct but does not apparently receive any cells therefrom. When it reaches the urinogenital sinus it fuses with its wall at the point which represents the old cloacal horn. The epithelium of this horn meanwhile has proliferated until it has filled the lumen and converted the horn into a solid cord separating both the müllerian duct and the wolffian duct from the cavity of the urinogenital sinus. Of course, the wolffian duct cannot function at all after it loses communication with the cavity of the sinus, and the retrogression of the mesonephros thereafter is rapid. The solid sinus cord later becomes the caudal third of the lateral vaginal canal which (as Hartman, '23, has shown) remains uncanalized until just before the first prooestrum. The müllerian duct forms the cranial two-thirds of the vagina. This is the situation which Buchanan and Fraser ('18) found in Trichosurus, and which Hill and Fraser ('25), on the basis of an histological study of the adult, predicted would be found in Didelphys.

To revert now to the 1-week-old opossum, in addition to having a functional metanephros and the beginning of a müllerian duct, it also has the beginning of a tectorial membrane in the inner ear, and the cochlear duct has grown out to one and one-half turns (fig. 63). The lagena or apical tip is still actively growing. The vestibular part is just completing the growth which will soon separate it from the sacculus everywhere except at the very slender canalis reuniens. The only part of the duct which has already ceased to grow is the second half of the first turn. This region which ceases to grow first gets a 'head start' in differentiation, and the first appearance of the tectorial membrane is at this point. The differentiation of other structures in the inner ear will follow this same course, that is, each new structure appears first in the apical part of the first coil and later spreads in both directions from this point.

About 1½ weeks after birth sex becomes distinguishable externally on account of the appearance of the pouch rudiments in the female and the scrotal rudiments in the male. Both pouch and scrotum are represented at first by paired bilateral folds. The position of the scrotal folds in the male corresponds to the position of the posterior end of the pouch folds in the female. Just as in the female the posterior ends of the marsupial folds approach each other and fuse to form the posterior part of the pouch at a point just cranial to the clitoris; so in the male the scrotal folds approach each other and fuse to form a scrotum just cranial to the penis (fig. 64). In later stages both the pouch and the scrotum migrate further craniad so that they are finally situated a considerable distance anterior to the clitoris and penis respectively (fig. 5).

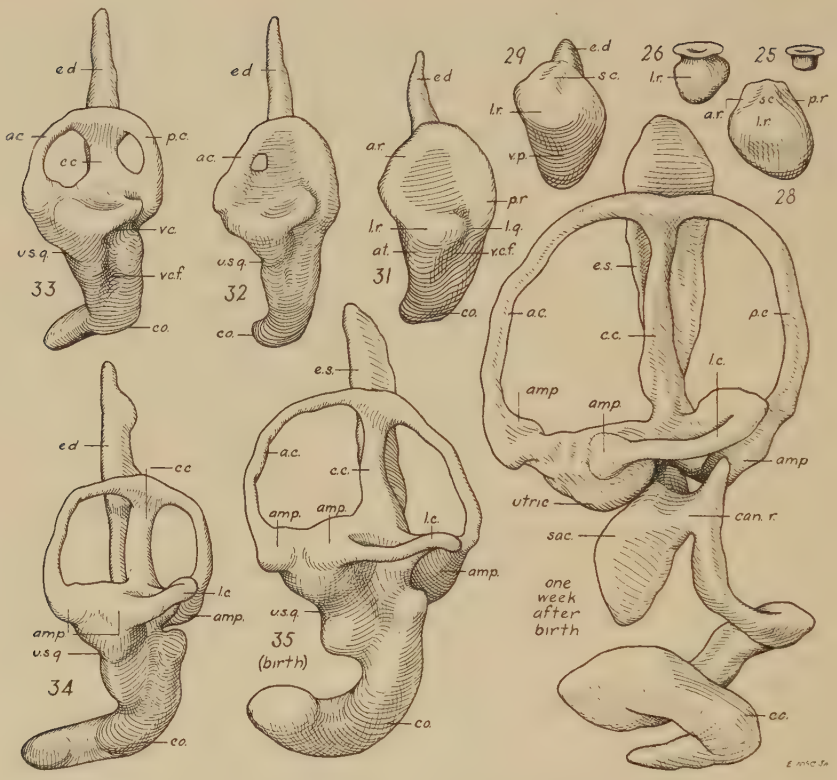

Fig. 63 Lateral view of reconstructions of inner ear from stage 25 until 1 week after birth (from Larsell, McCrady, and Zimmermann, '35).

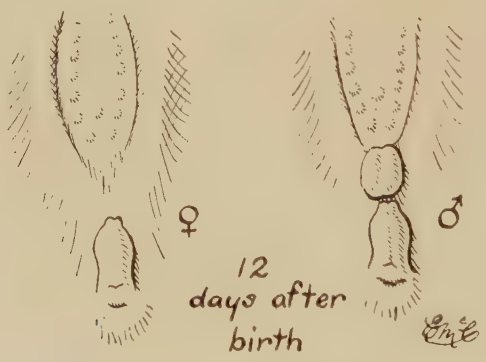

Fig. 64 Rudiments of pouch and scrotum 12 days after birth.

This perfect parallelism between the development of the scrotal anlagen and the lips of the pouch makes it impossible for the present author to avoid the conclusion that the lips of the pouch represent the labia majora, the homologues of the scrotum in higher mammals. The situation of the labia majora in this case some distance anterior to the clitoris is certainly no more surprising than the situation of the scrotum at the same distance anterior to the penis. The fact that this homology has apparently never been suggested before, though a number of investigators have studied the development of the pouch and the descent of the testes, suggests that there must be some obvious and fundamental objection to it; but I confess I have been unable to think of any such objection.

Nerve endings appear in the cochlear duct and in the vestibular parts of the inner ear about 4 weeks after birth, but neither the acoustic nor the vestibular apparatus is functional at this time. Vestibular reflexes appear about 6 weeks and acoustic reflexes about 7 weeks after birth. The range of hearing is at first restricted and sensitivity is poor. As sensitivity improves, the range of hearing extends to higher and lower notes. The first notes heard are around 1500 c.p.s. which is about the center of the adult opossum's range.

The 50-day-old opossum is about the size of a mouse, and has a short and sparse coat of fur. Sometime between then and the sixtieth day it opens it eyes and mouth, and for the first time since birth is free to crawl about on its mother's body. It cannot yet control its body temperature, however, and does not leave its mother. Also it is not yet weaned. It must continue to nurse for some 30 more days, and it returns frequently to the pouch for this purpose. If separated from its mother at this time, its body temperature soon goes down approximately to room temperature, and it goes into a sort of coma resembling hibernation.

By the time it is 70 days of age the young can control its body temperature, can hear five or six octaves, and can venture on slight excursions away from its mother; but it still returns to the pouch for food. By 80 days it has become the size of a large rat. The mother's pouch has enlarged enormously to accommodate the growing young, but it cannot hold a large litter of 80-day-old specimens. Accordingly special arrangement has to be made for the last part of the nursing period. This arrangement is that the mother's nipples elongate to some $1\frac{1}{2}$ inches, which makes it possible for the young to lie outside the pouch while they nurse.

The first tooth to erupt is the deciduous third premolar, which appears at about 60 days of age and has a curious molariform shape. It functions as a molar until sometime between the ninetieth and the three-hundredth days when it is shed and replaced by a permanent

premolar. With this one exception the milk dentition is abortive—that is to say, the other deciduous teeth do not erupt, but remain as functionless vestiges until a comparatively late stage. The second tooth to erupt belongs to the permanent set. It is the second premolar and is present at 75 days. Between the seventy-fifth and eighty-fifth days the last four incisors, the canine, and the first premolar, erupt. By the ninety-fifth day the first molars and the first incisors are present, and only the last three molars and the third premolar remain to be cut.

With teeth ready for solid food, the young are ready to be weaned. This usually occurs between the eightieth and the ninetieth days. At this time the animal has developed his adult auditory range—about eight octaves (McCrady, Wever and Bray, '36). Curiously enough, the notes he hears best from this time on (about 7000 c.p.s.) are not at the center of his range, but are decidedly nearer the high end. And this fact is probably responsible for the fact that he can be startled more violently by rustling leaves or hay than he can by talking or even loud shouting. The sounds made by the rustling of leaves are composites of short wave lengths, and are much louder to the opossum's ear than they are to ours.

The sound which the young makes when separated from the mother is also a composite of short wave lengths and is probably very loud to the opossum's ear.

Very soon after the mother has weaned a litter and ceased to lactate, she goes into another oestrus. I have seen a female copulate on the second day after she was separated from her litter. She produced a new litter 13 days later.

Some experimental techniques. It is hoped that one of the uses of this description of normal stages of opossum development will be to serve as a background for various experimental studies. In this connection it may be of interest to mention some of the experiments which have been performed on embryos and pouch young in this laboratory to indicate the type of procedure employed and the suitability of the animal for operative work.

The only experiment with embryos so far is the preliminary study of the behavior of specimens of stages 33 and 34 already described on page 182. The advantageous feature of the opossum embryo for such studies is that it has no placenta, and correspondingly, can be removed from the maternal uterus without the ligation or section of any part of its circulatory system.

On specimens of stage 35 two operations have been performed. The lens vesicle of the eye has been removed, and the limbs have been amputated. In neither of these cases was any anaesthetic employed on the pouch young. If the mother was not too excited she also was

not anaesthetized. My assistant laid her on her back on the operating table, held the back of her neck with his left hand, and held her pouch open with his right. This is the simplest procedure, and the most desirable when possible; but if the mother becomes too excited, as is sometimes the case, she must be anaesthetized. We have usually used an ether cone for this purpose. If any of the ether passes through the mammary gland into the milk, it is not enough to have any recognizable effect on the young in the pouch.

In the removal of the lens from the eye the epitrichium between the lids was cut with a small pair of spring scissors the tips of which had been specially ground to a very fine point. Then a small sliver of steel broken from the edge of a thin safety razor blade, and held in a small needle chuck, was used as a knife for slitting the anlage of the cornea. When this was cut, gentle pressure on the side of the optic cup with the tip of a dull probe, caused the lens vesicle to pop out. If the original incision through the epitricium is along the line of fusion of the lids, there is no bleeding in this operation. The incisions were allowed to close by themselves.

In the amputation of the legs three methods were tried by Dr. Olof Larsell and myself, and only one method found satisfactory. Cauterization and simple snipping off with fine scissors both lead to high mortality. On the other hand, if a loop of fine, white, cotton thread is tied tightly about the limb, the portion distal to the ligature can be cut off immediately or can be left until the following day and then cut off. Survival in this case is usually excellent, and not even a scar can be found on the stump at later stages. Occasionally the ligature slips, or is not made tight enough to begin with, allowing blood to get into the limb. In several such cases the result was hemostasis (due apparently to occlusion of the veins, which are superficial, but not the arteries, which are deep), with great swelling and discoloration of the limb and eventual death of the individual so affected. Both hind limbs can be amputated at one time without danger, but when a hindlimb and forelimb were removed from the same individual it was found advisable to amputate one at a time, with an interval of 24 hours for recovery after the first operation.

Though regeneration was not the subject of these studies, it may be of interest to note that none occurred in any of these cases. At the time of the operation the hindlimb was only an embryonic paddle with small digital buds, and the opossum is more primitive than any mammal the embryo of which has hitherto been tested for regeneration.

Both of these operations were done with the young still attached to the mammary nipple. In fact, this attachment was found to be a convenient mechanism for immobilization of the head. The rest of the body was immobilized for the eye operation by means of a strip of adhesive tape fastening the young to the mother's abdominal wall.

When the operations are performed less than 4 days after birth, it is fairly common for the young to become detached and fall out of the pouch. After about a week no such danger is encountered—even if the specimen dies, he remains attached to the nipple. From this time until about 45 days after birth, if the young is forcibly removed from the nipple, he is usually unable to re-attach, and so dies. After about 45 days they can be removed without danger of tearing the lips, as the mouth is nearly ready to open spontaneously, and when replaced in the pouch the young will resume nursing.

In connection with studies of the development of hearing (done in collaboration with several others) I have operated upon pouch young between 48 and 80 days of age in order to place an electrode upon the

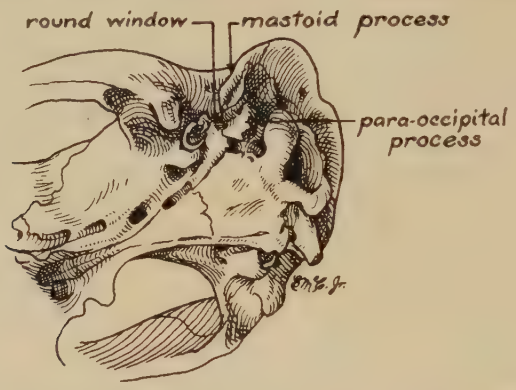

Fig. 65 Ventrolateral view of posterior part of adult skull (from McCrady, Wever and Bray, '37 a).

round window for the purpose of observing the Wever and Bray phenomenon. The operative procedure is rather different from that employed on placental mammals, because in the opossum the squamosal, petrosal, and tympanic bones do not fuse to form a single temporal bone, but remain permanently separate as in reptiles (fig. 65). Accordingly, the middle ear is not encased in bone. In adults prolonged muscular traction has caused the mastoid portion of the squamosal bone to elongate and slightly overhang the round window. For this reason, animals less than 100 days old are preferable for this operation.

Figure 66 shows the operative field in a specimen 78 days old. The following summary of the operative procedure is taken from McCrady, Wever and Bray ('37):

After anaesthetization with Avertin, injected intraperitoneally in doses of 0.0001 cc. per gram of body weight a skin incision some $\frac{5}{8}$ inch long was made in the direction indicated in this figure. With a long, slender, pointed scalpel the loose connective tissue behind the facial nerve and immediately ventral to the mastoid process of the squamosal bone was then trimmed away. The sterno-mastoid muscle is continuous with the trapezius from their common origin on the mastoid process down to the point where the great auricular nerve comes between them. It was sometimes necessary, particularly in the older specimens, to cut away the muscle tissue for a few millimeters at this common origin to expose the endodermal lining of the middle ear. The exact point at which the endoderm was then cut is caudal to the exit of the facial nerve, ventral and as close as possible to the mastoid process, and dorso-cranial to the origin of the digastric muscle. Little harm can come from cutting somewhat too far caudad,

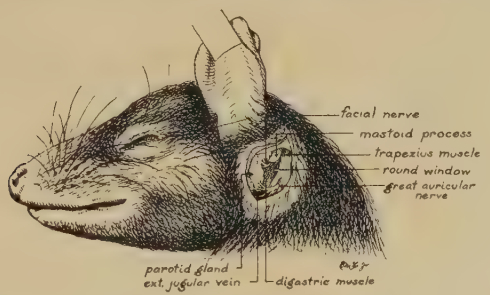

Fig. 66 Middle ear operation in 78-day-old opossum (from McCrady, Wever and Bray, '37 a).

as the paraoccipital process (fig. 65) limits digression in that direction; but it is important not to cut too far craniad as the tympanum and ossicles may be injured. The best limiting landmarks in the cranial direction are the exit of the seventh nerve and a small tubercle on the petrosal bone . . . . seen in figure 65 below and slightly to the left of the round window.

My former conjecture that this tubercle "probably represents the styloid process of higer mammals" was quite wrong. The attachment of the hyoid cartilage to the periotic capsule at the end of the embryonic period (fig. 61) is dorsocaudal to the stapes and the fenestra ovalis instead of ventrocranial to the fenestra rotunda, and there is no indication of this tubercle at this time. The latter arises much later as a process of the petrosal bone not associated in any way with the hyoid cartilage. It provides a surface for the attachment of the caudal border of the petrosal-alisphenoid ligament which forms part of the ventral wall of the middle ear cavity.

After a similar surgical approach I have used a very small dental drill to destroy parts of the cochlear duct (McCrady, Wever and Bray, '37). After the lesion had been made in the inner ear a few dermal sutures were made and the deeper structures were left to take care of themselves. The sutures were sometimes covered with thin celloidin. Aseptic precautions were observed throughout the operation, and healing was found to be very rapid. Survival was almost 100%. From 1 month to 3 months after the first operation a second operation was performed for the purpose of making electrical tests of the experimental ears. A comparison of the range of hearing before and after such operations is in progress.

The evolution of the mammals. One cannot examine a large number of embryos of any species without pausing from time to time to reflect upon the subject of evolution. The study of a particularly primitive mammal leads one almost irresistably to the subject of mammalian phylogeny.

The theory which has been the basis for all discussions of this subject in modern times was published by Prof. T. H. Huxley in 1880. He divided living and extinct mammals into three great subclasses—the Prototheria, the Metatheria, and the Eutheria. Of these, he conceived that the Prototheria in an unspecialized form evolved first. They then split into two branches. One branch by a relatively slight departure from the original stem became the modern Monotremata. The other gave rise to the Metatheria, which in turn split. One group of Metatherians by relatively slight specialization became the modern Marsupials; the other gave rise to the Eutheria. So in the modern world we have somewhat specialized representatives of all three of the ancestral stocks—the Monotremes are slightly specialized Prototherians; the Marsupials are slightly specialized Metatherians; the Placentalia or Monodelphia are Eutherians in various degrees of specialization.

This has been, and is today, the most widely accepted theory of the evolution of the mammals; but it has met with considerable opposition—principally as a result of the discovery of many supposedly Eutherian characters among the modern marsupials. Such discoveries have led a few to believe that the modern marsupials are a degenerate group descended from Eutherian ancestors (e.g., Dollo, 1898, and Hubrecht, '08).

Wilson and Hill (1897) and Hill (1897), while not accepting that terminology, that is to say, not granting that the marsupials are descended from Eutheria, at any rate, feel that they are descended from a stock which was both diphyodont and placental. In other words, they believe that the Metatheria developed a double dentition and an allantoplacenta before they split up into the Marsupialia and Eutheria.

Bensley ('01 a, '01 b, '03) points out that as far as the diphyodont condition of the Metatheria is concerned, that is exactly what Huxley hypothesized in his original paper (1880, p. 655); and it seems to be well substantiated by the finding of a suppressed milk dention among modern marsupials. But as to the placental condition, only one marsupial is known to have an allantoplacenta comparable to that of Eutheria, and that marsupial, Perameles, is one of the more specialized forms, not a very primitive one. Accordingly, he thinks it more likely that this is a case of evolutionary convergence.

Flynn ('22) argues that the extraordinary similarity of the allantoplacenta in Perameles to that in the Monodelphia makes convergence seem an inadequate explanation, and that all of the aplacental marsupials (i.e., most of the marsupials) must be degenerate in this respect, not primitive. This seems unsound for two reasons. There are too many other instances of striking convergence between the marsupials and the placental mammals; and there are too many other anatomical indications that the most primitive marsupials are aplacental. In a similar way one might argue that the extraordinary similarity between the incisors of the Diprotodontia and those of the Rodentia proves that the marsupials are derived from rodent ancestors, and that all of the Polyprotodontia are degenerate, not primitive.

Striking examples of homoplasy or convergence are not to be wondered at among members of a single Phylum and Class. If the eye of a mollusc is found to be identical in almost every detail with the eye of a vertebrate, one may justifiably express consternation; but among animals universally believed to have had common ancestors at a not very remote geological period, this kind of convergence is not only natural, but common. The detailed parallelism between the adaptive radiation of the Australian marsupials and that of the monodelphia, which has been known ever since the days of Cuvier, furnishes numerous instances in point. Witness the extraordinary similarity between the marsupial mole and the eutherian mole, the marsupial rodent and the eutherian rodent, the marsupial cats and the eutherian cats, the flying phalangers and the flying squirrels, etc.

In regard to the evidence that the most primitive marsupials are aplacental, we may ask what features we may regard as indications of primitiveness. If we take the same criteria which have been used in working out the evolutionary tree of the Eutheria, we can establish a very similar tree for the marsupials.

Dollo (1899) showed very convincingly that the feet of all modern marsupials can be derived from an ancestral form very remarkably like that of the modern opossums (Didelphyidae), but that even this primitive foot pattern included one specialized feature—the hallux of

the hind foot is opposable, an arboreal adaptation. This is in line with Huxley's theory that one of the distinctive features of the marsupials when they first emerged from the primitive Metatheria was the assumption of an arboreal habit. So this evidence points to the opossum, an aplacental marsupial, as near the bottom of the evolutionary tree, and Perameles, with its enlarged fourth digit, reduced hallux, and syndactylous second and third digits, as one of the more specialized forms.

Bensley showed that the teeth of the American Didelphyidae are nearer the original stem than are those of any other living marsupials; and the "The molar tooth patterns of the stem form are almost exactly reproduced in those of the Oligocene Opossums (Peratherium)" ('01 b, p. 249). Both in number of teeth and in elaboration of the molars, Perameles is more specialized than is the opossum.

Brass (1880) showed that the most primitive form of the vaginae is found in the Didelphyidae and that every gradation can be found between this form and the peculiar median vagina in the specialized Macropodidae.

Wallace (1876) pointed out that during the Tertiary period in Europe there were no other marsupials than the Didelphyidae, and Lydekker (1896) considered the most primitive Australian marsupials, the Dasyuridae, to be descended from the Oligocene Didelphyidae of the Northern Hemisphere.

All these lines of evidence converge to indicate that the aplacental opossums are the most primitive living marsupials, and that the nicely graded series which Hill (1897) pointed out between the allantoplacenta of Perameles, the allantois in contact with the serosal membrane, but not forming a placenta (Phascolarctus and Aepyprymnus), and the small allantois enclosed in the yolk sac (Didelphys), probably indicates an evolutionary line in the opposite direction from that inferred by him. That the non-placental allantois is among marsupials the more primitive, is also suggested by its widespread distribution (Didelphyidae, Phalangeridae, and Macropodidae). In the light of the development of the respiratory allantois in the Sauropsida, there can be no doubt about the fact that the opossum's allantois is degenerate; but the evidence indicates that it has degenerated from the sauropsidan condition, not from the eutherian. The early birth and precocious functioning of the lungs in marsupials removes the need for a respiratory allantois. The opossum's lung begins to function at a much earlier stage of general body development than does that of the chick (see chapter IX, p. 90). With its respiratory function usurped by the precocious lung, it is not surprising that the allantois of most marsupials is to some extent degenerate.

Bensley ('01 a) has also pointed out the following facts about the mammary apparatus and the pouch. The observation of Gegenbaur that the mammary glands of monotremes are specialized sudoriparous glands whereas those of marsupials and placentals are of sebaceous origin is sometimes cited as evidence that the marsupials are derived from the Eutheria, not from the Prototheria. Bensley quotes Gegenbaur (1880, p. 35) to the effect that he found some sebaceous milk glands even in the monotremes and he recognized the probability that in the original prototheria both sorts of glands were present. In the modern monotreme one type has been emphasized, so to speak, and in higher mammals the other. Klaatsch (1892) found both sorts present in the artiodactyl ungulates, which confirmed this interpretation.

Klaatsch also recognized the homology of the tubular teat cavities of the artiodactyl ungulates and the circular folds which surround the nipples in marsupial embryos. He was of the opinion that the pouch of the marsupials is formed by the fusion of these teat cavities, and correspondingly he argued that the ungulates could not have passed through a marsupial stage. Bensley mentions that this is beside the point, as Huxley had regarded the pouch as a character of modern marsupials, and had suggested that the Eutheria were derived, not from the marsupials, but from the primitive Metatheria. Furthermore I am dubious about these teat cavities having anything to do with the formation of the pouch. If the theory of pouch formation that I suggested in a previous section of this paper be correct, then the primitive Metatheria must have had paired, longitudinal, labioscrotal folds, and from these are derived both the pouch of the marsupials and the labia majora of the placental mammals. In this feature, as in most others, the modern Didelphyidae and Dasyuridae would be nearer the original ancestral stock than any other living marsupials.

Lest in this emphasis on a few debatable points we lose sight of the myriad anatomical features in which the marsupials are nearer the Sauropsida than are the placental mammals let me call to mind again: the presence of both ducts of Cuvier; the absence of a septum secundum in the heart; the absence of a corpus callosum between the cerebral hemispheres; the separate squamosal, petrosal, and tympanic bones; the presence of a complete coracoid cartilage in the embryo. And then we may add a few which have not been mentioned before in this paper.

The cells of Paneth are not confined to the glands of Lieberkühn, but are distributed all over the surface epithelium of the intestine, and are primitive in structure. This is like the condition in the lizard, and unlike that of placental mammals (Klein, '06). The

tapetum lucidum of the eye is in the pigment layer instead of in the chorioid, and is composed of crystals of guanin instead of fibrous cells. This is like the condition in the crocodile family, and in contrast to the condition in the ungulates and carnivores (private communication from G. L. Walls). The subintestinal vessel is a vein as in lower vertebrates, not an artery as in all placental mammals (Kimball, '28). The capillaries of the cerebral cortex form simple loops between arterioles and venules instead of being plexiform as in higher mammals. This is like the condition in amphibians and reptiles (Wislocki, '37).

It is difficult to imagine that all or even a large proportion of these features are the result of degeneration from a eutherian condition It seems much more reasonable to interpret them as evidence that the marsupials have diverged less from the original common stock than have the eutheria. Everything seems to bring us back to Huxley's original theory essentially unscathed. The only modification of it which seems possible is the one advocated by Wilson and Hill, and Flynn—that the metatheria acquired an allantoplacenta before they gave rise to the marsupials and the eutheria. This is possible; but neither compelling, nor, I think, likely.

One point on which almost all the evidence is in agreement is that the Didelphyidae are the most primitive marsupials alive today, and that fact adds another source of interest to the embryology of the Virginia opossum.

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In response to numerous requests from this country and abroad for an opossum bibliography, it has been considered desirable to bring together at this place a rather comprehensive list which may be of wider interest than the volume to which it is attached. The references listed below, therefore, are not all cited in the text. Many deal with subjects other than embryology. A few are concerned with related animals, not with the opossum itself. Fewer yet refer to animals not even closely related, but yielding data which throw light upon some observation or principle referred to in the text.

The original list compiled by the author included a much larger number of titles. It has been contracted to the present size by the elimination of those which could not be consulted on account of inaccessibility of the journal, or incompleteness or incorrectness of the reference data; those which deal with distantly related marsupials a knowledge of which is not very pertinent to anything discussed in the text; and finally, a few which are apropos in subject matter, but so superficial or incorrect as to be considered more disconcerting than useful. This last category of eliminations might have been made more extensive, but the author was anxious to err in the direction of over-comprehensiveness rather than of unduly personal selectiveness.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

67 Normal embryonic stages 1 to 13.

1 One-celled ovum.
2 Two-celled ovum.
3 Three-celled ovum.
4 Early 4-celled ovum with large blastomeres.
4′ Late 4-celled ovum with small blastomeres.
5 Six-celled ovum.
6 Eight-celled ovum.
7 Twelve-celled ovum.
8 Sixteen-celled ovum.
9 Thirty-two-celled unilaminar blastocyst.
10 First endodermal mother-cells.
11 Earliest medullary plate and considerable yolk in gastrocoele.
12 Expanded and attenuated vesicle with endodermal cells still almost entirely under medullary plate.
13 Ellipsoid blastocyst eccentric in albumen and with endodermal cells spreading toward equator.

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PLATE 2

EXPLANATION OF FIGURES

68 Normal embryonic stages 14 to 23.

14 Spherical bilaminar blastocyst.
15 Clear spot in medullary plate.
16 First mesodermal cells forming cloud in the clear spot.
17 Primitive streak with mesodermal crescents.
18 Hensen's node.
19 Primitive groove. Mesoderm still only beneath medullary plate.
20 Clear spot in front of Hensen's node. Mesoderm extending beyond medullary plate.
21 Notochord equal to or shorter than primitive groove.
22 Notochord longer than primitive groove. First somites forming.
23 First coelomic rudiments. No heart. No optic cups.

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PLATE 3

EXPLANATION OF FIGURES

69 Normal embryonic stages 24 to 35.

24 Optic cups but no contact of medullary folds.

25 Contact of medullary folds, but no proamniotic fold.

26 Proamniotic fold, but no caudal amniotic fold.

27 Caudal amniotic fold, but anterior neuropore and otocysts still open.

28 Anterior neuropore and otocysts closed. Amniopore still open.

29 Amniopore closed. Forelimb becomes a club.

30 Forelimb paddle. Secondary lumbar flexure.

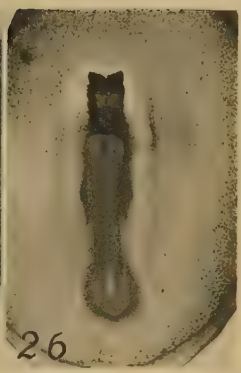
31 Diameter of allantois approximately one-third length of body. Hind limb bud. Frontal process.

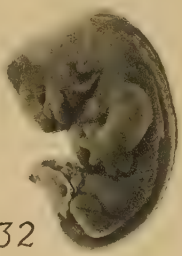
32 Diameter of allantois approximately two-thirds length of body. Hind limb club.

33 Diameter of allantois equal length of body. Hind limb paddle. Eyelid folds.

34 Oral shield. Epitrichium covers eyes, ears, and sides of mouth. Claws on forelimb digits.

35 Oral shield practically resorbed. Umbilical cord lost or very much constricted.





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THE MAMMALIAN VENA CAVA POSTERIOR

AN ONTOGENETIC INTERPRETATION OF THE ATYPICAL FORMS OF VENA CAVA POSTERIOR (INFERIOR) FOUND IN THE ADULT DOMESTIC CAT (FELIS DOMESTICA) AND IN MAN

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FORTY-SIX PLATES (SIXTY-THREE FIGURES)

The material presented in this Memoir by Professor Charles F. W. McClure is the result of the joint investigation of Professors Huntington and McClure prior to 1920.

[1] Deceased, January 5, 1927.

MEMOIR 15

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I. FOREWORD

The atypical condition of the vena cava posterior (inferior) often found in adult man and other mammals has for several decades been a matter of much interest to anatomists, and on this subject there has sprung up an extensive literature. In the late nineties, I began a study of this problem; and in 1905, Doctor Huntington became associated with me in a detailed investigation of the subject. The cases described in the literature before this time, as well as those which Doctor Huntington and I had separately observed, presented a wide range of atypical forms which neither of us could satisfactorily explain. It now appeared to us that since an atypical condition in the vena cava of an adult signifies a deviation in development from the typical plan, its ontogenetic interpretation would be found in the detailed study of the development of all embryonic vessels which had entered into the formation of the venous system. Accordingly, we set about making a joint investigation of the development of veins in the cat, as most of the atypical conditions observed by us were to be found in the variants of this animal. During the course of our work we became involved in the problem of the genesis of the lymphatics, and in consequence the publication of our joint paper on the development of the veins in the cat was delayed until 1920. It is largely due to Doctor Huntington's prolonged illness, dating from about 1918, and his death in 1927, that the publication of this memoir has been delayed to the present time.

The basic material presented in this memoir is the result of our joint work, accomplished before Doctor Huntington's illness. Our purpose has been to illustrate and to interpret from the standpoint of their development all known atypical forms of vena cava posterior. We have constructed a composite ontogenetic diagram of the embryonic venous system, on the basis of which we have classified under seventeen main

Types the atypical or variant conditions of the vena cava, actual as well as potential, in the adult cat. Furthermore, not only have we interpreted all the atypical forms of the cava already known, but also we have predicted the existence of others, some of which, since the publication of our first papers, actually have been reported by other investigators as found in the adult cat and in man.

In the following pages I have presented the results of our investigation in its original intention. While in recent years I have been without the advice and wise counsel of my colleague, I trust, nevertheless, that I have been able to do full justice to our subject, and that I have carried to a successful conclusion the work projected in 1905, continued by us jointly through fifteen happy years, and brought to date and finished by me since Doctor Huntington's illness and death.

CHARLES F. W. McCLURE.

II. THE NORMAL AND THE ATYPICAL VENA CAVA POSTERIOR (INFERIOR)

The explanation and interpretation of the atypical conditions of the vena cava posterior encountered by us in an extensive series of adult examples of Felis domestica are fully to be found in the comprehensive system of embryonic venous channels normally functioning in different ontogenetic stages. The acquisition of what is regarded as the normal type of vena cava in the cat is contingent on the selection and the continued development in the majority of individuals of certain venous pathways into the adult stage. The average individual thus assumes the venous type considered normal for the species. Any deviation from this plan is to be regarded as an atypical condition or variant. It is brought about by the selection and continued development of other existing embryonic channels which normally are not carried into the adult stage.

The vena cava posterior in any one of its atypical forms has usually been described by anatomists as an 'abnormality.' In the sense that an atypical condition is not normally encountered in the adult, such a designation is correct. On the other hand, all atypical cavae mentioned in the following pages are normal rather than pathological, in the sense that they are derived from vessels which during the course of ontogenetic development have normally functioned in the embryo.

III. THE SUPRACARDINAL SYSTEM OF VEINS

In 1905, when we first began a comprehensive study of the development of veins in the cat, it was generally supposed that the posterior cardinal vein of the embryo entered directly into the formation of the lumbar portion of the vena cava posterior. The acceptance of this view was due to the brilliant pioneer work of Hochstetter ('93), whose figures illustrating the development of the veins are well known to all anatomists. While we recognize the importance of Hoch-

stetter's investigations, they are nevertheless lacking in details sufficient to explain all atypical conditions observed in the adult mammal, especially in the cat. What we found to be the case is that the posterior cardinal vein, as such, does not, as has hitherto been supposed, enter into the formation of the cava in the lumbar region of the cat. The dorsal limb of the periureteric venous ring from which, according to Hochstetter, the lumbar cava also is in part formed, does not split off from the posterior cardinal, but belongs to a separate and independent system of veins which we have designated the *supracardinal system of veins.*

In 1907,[2] we described the supracardinal system of veins as follows:

> A bilateral and originally symmetrical venous channel develops dorsomedial to the primitive postcardinal vein by longitudinal anastomoses between somatic postcardinal tributaries. This secondary vein channel forms what we have termed the *supracardinal system of veins.* It extends from the level at which the posterior limb veins open into the postcardinals to a point cephalad where it joins that portion of the postcardinals which alone persists to form the anterior end of the adult azygos. Between these levels the supracardinal enters into the definite organization both of the adult postcava in its postrenal division and of the azygos in its lumbar and part of its thoracic segments, entirely replacing in these districts the primitive postcardinal veins. The postrenal division of the adult postcava is formed as the result of a secondary median fusion by means of transverse anastomoses dorsal to the aorta of the postcaval portions of the supracardinals, the primitive postcardinal veins not entering into the formation of this portion of the main trunk of the adult vessel.

IV. A RECONSTRUCTION OF THE AXIAL VEINS IN THE LUMBAR REGION OF A 16-MM. CAT EMBRYO

It is not the purpose at the present time to describe again, in detail, the development of veins in the cat. This we have already done in a paper published in 1920, to which the reader is referred.[3] The chief object which the present publication has in view is to bring together all atypical forms of the

[2] Am. Jour. Anat., vol. 6 (Proc. Ass. Amer. Anat.).

[3] Anat. Rec., vol. 20.

vena cava posterior which have been observed by us and by others, especially in adult man[4] and the cat, and to show how they may be interpreted as modifications of a normal ontogenetic venous plan. Such a composite ontogenetic plan of the veins is shown in figure 1. Here are represented the main axial venous channels and their anastomoses, which, in the cat, make their appearance at one time or another during the course of ontogeny. Under normal conditions certain of these veins function only temporarily and then disappear, while others are carried into the adult stage. Atypical conditions of the veins are brought about by the persistence of some one or more of the embryonic channels which normally are not carried into the adult stage.

Before considering the composite ontogenetic plan of the veins as depicted in figure 1, we will examine a typical reconstruction of the veins of the cat, the basis upon which in general the composite diagram (fig. 1) is built. Such a reconstruction is represented in figures 2 and 3, which show the main axial venous channels in the lumbar region on the right (fig. 2) and the left (fig. 3) sides of a 16-mm. cat embryo. These axial vessels consist of the right and the left supracardinals (*Sprc., B* and *C*) and the right and the left posterior cardinal veins (*Pc., A* and *D*). At an earlier stage of development both supracardinal and posterior cardinal veins extended as continuous vessels throughout the lumbar and the thoracic regions, so that blood from the caudal region of the body could pass directly to the heart through either the supracardinal or the posterior cardinal veins. The separation of these vessels into a lumbar and a thoracic division has been brought about by the formation of the unpaired prerenal division of the vena cava (*P.Hep., P.Sub.,* and *Int.Subc.Anast.*), through which, in the 16-mm. embryo, all blood collected by the lumbar axial veins is now returned to the heart. The prerenal division of the vena cava is joined by the axial veins

[4] The publication by McClure and Butler, in 1925, on the development of the vena cava inferior in man, has made it possible for the writer to confirm and to enlarge upon our previous observations.

of the lumbar region at about the level at which the embryonic renal veins (*R.V.*) are found. In this region, by extensive anastomoses, the veins almost completely encircle the aorta, forming a circumaortic venous ring, which we have designated the *renal collar*. In order to reach the prerenal division of the vena cava, blood in the lumbar supracardinals must pass, on each side of the body, through the lateral portion of the renal collar (*Subc.Sprc.Anast.*), and blood from the posterior cardinals (*Pc.*, *A* and *D*), which drain the gonads and the mesonephroi, reaches the prerenal division of the cava through the subcardino-posterior cardinal anastomosis (*Subc. Pc.Anast.*) without passing through the lateral portion of the renal collar.

On the right side of the embryo (fig. 2), a complete periureteric venous ring is present, through which the ureter (*Ur.*) passes. The ventral circumference of this ring is formed by the right lumbar posterior cardinal (*Pc.Dext.*, *A*), and its dorsal circumference by the right lumbar supracardinal vein (*Sprc.Dext.*, *B*). On emerging from the kidney, the ureter at first passes dorsal, and farther caudad, ventral to the posterior cardinal, lying between the aorta and this vein. On the left side of the embryo (fig. 3), no periureteric venous ring is present. Its absence is due to the loss of a caudal connection which previously existed between the left lumbar supracardinal (*Sprc.Sin.*, *C*) and the left lumbar posterior cardinal vein (*Pc.Sin.*, *D*). The relations which the ureter bears to the posterior cardinal and the supracardinal veins remain the same, however, on the left as on the right side.

The posterior cardinal veins join the renal collar at its ventrocaudal border and collect blood from the mesonephroi and the sex glands (gonads). With the disappearance of the mesonephroi, the posterior cardinal veins retain their connection with the gonads through the subcardinal veins and, together with the subcardinals, form the internal spermatic veins of the adult.

It is evident, for the most part, that in the 16-mm. cat embryo the veins of the lumbar region still retain their early bilaterally symmetrical plan—a condition which later becomes transformed into an asymmetrical one. This change is brought about chiefly by the loss and atrophy of vessels on the left side. For example, the vena cava posterior of the adult cat is derived from the following embryonic veins: the right lumbar supracardinal (*Sprc.Dext., B*), which retains, through a dorsal anastomosis, a connection with the left iliac vein; the right subcardino-supracardinal anastomosis (*Subc.Sprc. Anast.,* the right side of the renal collar); the right subcardino-posterior cardinal anastomosis (*Subc.Pc.Anast.*); the intersubcardinal anastomosis (*Int.Subc.Anast.*); a portion of the right subcardinal, the pars subcardinalis (*P.Subc.*), and the V. hepatica communis and the hepatic sinusoids (pars hepatica, *P.Hep.*). The lateral portion of the renal collar on the left side and the cranial half, at least, of the left lumbar supracardinal (fig. 3) undergo atrophy; but the caudal half of the left lumbar supracardinal appears to fuse with the right to form the caudal half of the adult caval vein.

With the atrophy of the left side of the renal collar (*Subc. Sprc.Anast.,* fig. 3), the left renal vein (*R.V.*) retains its connection with the left subcardino-posterior cardinal anastomosis (*Subc.Pc.Anast.*), which forms the proximal portion of the adult left renal vein. Into the latter the left posterior cardinal (*Pc.Sin., D,* fig. 3), with its gonad tributaries, opens, thereby establishing the adult condition in which the internal spermatic vein joins the left renal vein. On the right side of the embryo (fig. 2), the right embryonic renal vein (*R.V.*) retains its original connection with the right side of the renal collar (*Subc.Sprc.Anast.*), which forms a segment of the adult caval vein. The right posterior cardinal (*Pc.Dext., A*), with its gonad tributaries, also retains its connection with the renal collar at its ventrocaudal border, and owing to an elongation, in a craniocaudal direction, of the right side of the collar (*Subc.Sprc.Anast.*), the junction of the posterior cardinal with the cava is carried somewhat caudal to that of the right

renal vein. In this manner we can account for the difference which exists on opposite sides of the body, in the junction of the internal spermatic vein with the cava.

V. THE COMPOSITE ONTOGENETIC VENOUS PLAN OF THE CAT

We may now pass to a consideration of the composite ontogenetic plan of the veins (fig. 1). In this diagram are shown two important sets of vessels not represented in the reconstruction of the veins in the 16-mm. cat. These are the subcardinal and the cardinal collateral veins. In the 7- to 8-mm. cat embryo the subcardinals form a bilaterally symmetrical system of veins, which anastomose with the posterior cardinal veins and, ventral to the aorta, with each other. They receive blood from the mesonephroi and the sex glands. The cardinal collateral veins, which are not shown in their entirety in the diagram, reach the height of their development at approximately the 29-mm. stage, and for this reason were not evident in the 16-mm. cat. It is also to be noted that, except for its cranial end, the thoracic division of the posterior cardinal veins has not been shown in the diagram (fig. 1).

If we compare figure 1 with figures 2 and 3, we shall see that in none of its fundamental aspects does the composite plan of the embryonic veins exaggerate the condition actually observed.

If we examine the composite ontogenetic venous plan of the cat (fig. 1), we observe that three pairs of longitudinal bilaterally symmetrical venous channels are represented in the lumbar region and that they consist of the posterior cardinals (*A* and *D*), the supracardinals (*B* and *C*), and the subcardinals (*Subc.*) into which the sex veins (Vv. spermaticae, *S.V.*) open. It is to be observed that the supracardinals anastomose with the posterior cardinals (*Pc.Sprc.Anast.*) and, *dorsal* to the aorta, with each other (*Int.Sprc.Anast.*); and that the subcardinals anastomose with the posterior cardinals (*Subc.Pc.Anast.*) and, *ventral* to the aorta, with each other (*Int.Subc.Anast.*). In addition to the three pairs of

longitudinal vessels, there is shown at the iliac level a median vein (*C.C.*) lying *ventral* to the aorta which, with contiguous veins, forms venous rings through which pass the umbilical arteries. This has been termed the cardinal collateral vein (*C.C.*, fig. 1), as it is a caudal component of a longitudinal subaortic system of veins, between the iliac and the renal levels, which, though transitory in the cat, in marsupials forms the vena cava posterior (McClure, '06). This subaortic plexus (*C.C.*, cardinal collateral system of veins) reaches the height of its development in the lumbar region of a 29-mm. cat embryo, as is shown in figures 4 and 5, representing, respectively, the right and the left sides of a reconstruction.

The veins at the renal level form a prominent circumaortic venous ring, which we have designated the renal collar (fig. 1). It is in this region that all the early bilaterally symmetrical longitudinal venous channels drain into the unpaired prerenal division of the posterior vena cava (*P.Subc.* and *P.Hep.*) which, from the renal collar, extends craniad through the liver to the right auricle.

The bilaterally symmetrical periureteric venous rings of early stages, through which pass the ureters, are accurately represented in the diagram (fig. 1). It is to be observed that as each ureter (*Ur.*) emerges from the kidney, it at first passes *dorsal* and then farther caudad *ventral*, to the posterior cardinal vein.

The supracardinal system of veins (*Sprc.*) is shown in the diagram (fig. 1) as a bilaterally symmetrical system of veins, extending as continuous vessels through the lumbar and the thoracic regions. In the thoracic, as in the lumbar region, the supracardinals anastomose freely with each other *dorsal* to the aorta, and it is from this intersupracardinal complex (*Int.Sprc.Anast.*) that the dorsally situated azygos vein of the adult is for the most part developed.

If the reader will take the trouble to master the simple conditions represented in figure 1, existing amongst the longitudinal venous channels and the anastomoses between them at different levels, he will have no difficulty in interpreting

the variant conditions presented by the veins in the adult cat or in man, to be described in the following pages.

As longitudinal channels, the subcardinals in the cat lose their identity at a relatively early stage of development, and the chance of their being retained in the adult in their embryonic form is negligible. With the exception of the inter-subcardinal anastomosis at the renal level (*Int.Subc.Anast.*, fig. 1), of the pars subcardinalis of the vena cava (*P.Subc.*), of a segment of the internal spermatic veins, and of the adrenal veins, the subcardinals are not otherwise carried into the adult stage.

The subaortic plexus in the lumbar region (cardinal collateral veins, *C.C.*, in figs. 4 and 5) develops late in ontogeny, in connection with the supracardinals, after the latter have assumed the function of returning practically all blood from the iliac veins to the prerenal division of the cava; therefore, the chance of these veins being retained in the adult is likewise very slight. The posterior cardinals, however (*A* and *D*, fig. 1), function as important vessels in conveying blood from the iliac region to the prerenal division of the cava until a relatively late stage of development (16- to 17-mm. cat embryo), and then are gradually replaced in performing this function by the supracardinal veins (*B* and *C*, in fig. 1). The persistence of the two posterior cardinals and the two supracardinals so late in ontogeny as conveyors of blood from the iliac veins to the prerenal division of the cava, accounts for the fact that some one, or else a combination of two or more of them, is often carried into the adult to establish an atypical condition. Most of the atypical forms of cava found in the lumbar region of the adult cat are due to the persistence of the right posterior cardinal, of the left supracardinal, of the left posterior cardinal, or of any one of the possible combinations which exist between the two posterior cardinal and the two supracardinal veins. As a matter of fact, there are in the lumbar region fifteen (15) possibilities of the persistence of one or more of these four veins. One of them constitutes the normal type of cava, while fourteen are atypical in character.

These *Types* are as follows (see *A, B, C,* and *D,* in fig. 1):

1. *Type A,* persistence of right posterior cardinal vein (*A*).

2. *Type AB,* persistence of right posterior cardinal (*A*) and right supracardinal (*B*) veins. Right periureteric venous ring present, through which ureter passes.

3. *Type ABC,* persistence of right posterior cardinal (*A*), right supracardinal (*B*), and left supracardinal (*C*) veins. Right periureteric venous ring present, through which ureter passes.

4. *Type ABCD,* persistence of right posterior cardinal (*A*), right supracardinal (*B*), left supracardinal (*C*), and left posterior cardinal veins (*D*). Right and left periureteric venous rings present, through which ureters pass.

5. *Type ABD,* persistence of right posterior cardinal (*A*), right supracardinal (*B*), and left posterior cardinal veins (*D*). Right periureteric venous ring present, through which ureter passes.

6. *Type AC,* persistence of right posterior cardinal (*A*), and left supracardinal veins (*C*).

7. *Type ACD,* persistence of right posterior cardinal (*A*), left supracardinal (*C*), and left posterior cardinal veins (*D*). Left periureteric venous ring present, through which ureter passes.

8. *Type AD,* persistence of right (*A*) and left (*D*) posterior cardinal veins.

9. *Type B,* persistence of right supracardinal vein (*B*) to form the lumbar division of the posterior vena cava. *Normal cava.*

10. *Type BC,* persistence of right (*B*) and left (*C*) supracardinal veins.

11. *Type BCD,* persistence of right (*B*) and left supracardinal (*C*), and left posterior cardinal veins (*D*). Left periureteric venous ring present, through which ureter passes.

12. *Type BD,* persistence of right supracardinal (*B*) and left posterior cardinal veins (*D*).

13. *Type C,* persistence of left supracardinal vein (*C*).

14. *Type CD,* persistence of left supracardinal (*C*) and left posterior cardinal veins (*D*). Left periureteric venous ring present, through which ureter passes.

15. *Type D,* persistence of left posterior cardinal vein (*D*).

With the exception of *Types* ABD, ABCD, BCD, and CD, all the atypical forms above mentioned have been observed in adult man or the cat either by us or by others. *Type* ABCD, however, has been figured by Hochstetter ('93) as occurring in Erinaceus europaeus.

There are three additional types in which the vena cava posterior may appear in the adult cat and in man in atypical form:

16. Persistence of embryonic supracardinal system of veins to form a continuous system of vessels in the lumbar and the thoracic regions, by means of which blood from the iliac region can be conveyed directly to the heart, without passing through the liver.

17. Persistence of renal collar (circumaortic venous ring at level of renal veins).

18. Persistence of subaortic plexus or cardinal collateral veins (*C.C.* in fig. 1). *Marsupial Type* of vena cava posterior.

Although for convenience of description we have classified the potential variant or atypical conditions of the vena cava posterior into the above-mentioned individual types, we recognize that in the same individual a combination of several types does occasionally occur. Such a classification, based upon a comprehensive ontogenetic plan of the veins, is a distinct advance over our former knowledge; it not only permits us to interpret the atypical forms of cava thus far observed, but also enables us to predict others which are potential and remain still to be found.

VI. THE ATYPICAL FORMS OF THE VENA CAVA POSTERIOR IN ADULT MAN AND THE CAT, INTERPRETED ON THE BASIS OF THEIR DEVELOPMENT FROM A COMMON GROUND PLAN, BY THE SELECTION OF POTENTIALLY AVAILABLE PATHWAYS IN THE EMBRYO

In the following pages there are given examples of atypical forms of the vena cava posterior thus far observed in both man and the cat. These atypical conditions can be interpreted on the basis of their development from a common ground plan, through the selection of potentially available venous pathways in the embryo. The variants described for man have been drawn from the anatomical literature dealing with this topic and from the extensive Huntington Collection in the College of Physicians and Surgeons of Columbia University. The adult material of the cat was in large part prepared by Professor William Darrach, in connection with the courses in mammalian anatomy in Columbia University. He examined 605 adult cats, and at the Twenty-first Annual Meeting of the American Association of Anatomists (December, 1906), reported the variations of the vena cava posterior and its tributaries, as shown by this series. His report is published in the Proceedings of the Association, Anatomical Record, no. 3, 1907. We wish here to express our deep obligations to Professor Darrach for his painstaking work, done largely in our behalf. Since the work of Professor Darrach, further extensive observations have been made by the writers in the Departments of Comparative Anatomy of Princeton and Columbia Universities.

As we have shown elsewhere, certain of these embryonic venous pathways which are not retained in the adult undergo atrophy, and the position formerly held by them is assumed by a lymphatic. This process has been termed by us the "extra-intimal replacement of a vein by a lymphatic." For a more complete understanding of the process involved, the reader is referred to our joint paper published in 1907,[5] in which this view was first advanced, and to papers published by us separately in 1910.

[5] Am. Jour. Anat., vol. 6 (Proc. Ass. Amer. Anat.).

We may now proceed to a detailed consideration of the atypical conditions of the vena cava posterior as found in the adult cat and in man.

1. Persistence of right posterior cardinal vein

Type A (*A* in fig. 1). Figures 6 and 7, cat (Darrach Series), and figure 8, man (Gladstone, '12)

A single main axial vein, to the right of the aorta, manifests itself as the persistent right posterior cardinal vein (vena cava posterior) by its relation to the right ureter, which lies at first dorsal to the vein, emerges between the latter and the right side of the aorta, and descends caudolateral over the ventral surface of the cava.

As shown in the reconstruction (fig. 2) and in the composite diagram (fig. 1), the right side of the renal collar (*Subc.Sprc.Anast.*) does not enter into the formation of the lumbar cava when in the adult the posterior cardinal vein (*A*) alone persists to form the cava. A vena cava posterior of *Type* A develops along the following pathways in figure 1: pars hepatica (*P.Hep*), pars subcardinalis (*P.Subc.*), intersubcardinal anastomosis (*Int.Subc.Anast.*), subcardino-posterior cardinal anastomosis (*Subc.Pc.Anast.*) and right posterior cardinal vein (*A*). In the examples of *Type* A represented by figures 6, 7, and 8, the iliac veins of the left side have established their connection with the cava (*A*, fig. 1) by the persistence of an anastomosis, dorsal to the aorta, between the main axial veins of opposite sides.

In figures 6, 7, and 8, the sex vein on the right side opens into the right posterior cardinal (cava) and on the left side, into the left renal vein. The anastomosis between the sex veins of opposite sides in figure 6 is due to the persistence of an embryonic intersubcardinal anastomosis (*Int.Subc.Anast.*, fig. 1), ventral to aorta, somewhat caudal to the renal level. Darrach observed this anastomosis in only two cases of his series of 605 adult cats. In *Type* A of the cat, the right sex vein (*V. spermatica*) is derived from the subcardinal plexus (*S.V.*, fig. 1) of the right sex gland, together with one (fig. 6)

or more (fig. 7) of the subcardino-posterior cardinal anastomoses (*Subc.Pc.Anast.*, fig. 1) caudal to the renal level, between the right subcardinal (*Subc.*) and the right posterior cardinal veins (*A*, fig. 1). The left sex vein of the cat is formed by the subcardinal plexus of the left sex gland (*S.V.*), by a left subcardino-posterior cardinal anastomosis (*Subc.Pc. Anast.Sin.*, fig. 1), and by a segment of the left posterior cardinal vein (*D*, fig. 1). At the renal level the latter joins the left subcardino-posterior cardinal anastomosis (*Subc.Pc. Anast.*) which, after atrophy of the left side of the renal collar (*Subc.Sprc.Anast.Sin.*), becomes the proximal portion of the left renal vein of the adult. Figure 5, which represents a stage of development in which the left side of the renal collar has completely disappeared (*Subc.Sprc.Anast.*), makes plain the composition and the relations of the left sex vein in the adult cat. Figure 5 should be compared with figure 3, in which is present the left side of the renal collar.

Type A was found by Darrach in twenty-two, or 3.6 per cent, of the 605 adults examined. It has been figured as occurring in the cat by Hochstetter ('93) and Gage ('98); in the cat and the rabbit by Graves and Davidoff ('22); and in man by Hochstetter ('93), Huntington ('07), Kolisko ('09), and Gladstone ('11).

2. Persistence of right posterior cardinal and right supracardinal veins. Right periureteric venous ring present, through which ureter passes

Type AB (*A* and *B* in fig. 1). Figures 9 and 10, cat (Darrach Series)

This interesting variant (figs. 9 and 10) shows a condition in which the right ureter passes through a periureteric venous ring. The dorsal circumference of this venous ring is formed by the right supracardinal vein; its ventral circumference, which receives the right sex vein, is formed by the right posterior cardinal. As shown in the composite diagram (fig. 1), the periureteric venous ring is due to the persistence of A and B; the common trunk cranial to the venous ring, between the ring and the renal veins (*R.V.*), has resulted

from an elongation of the right side of the renal collar (*Subc. Sprc.Anast.*). The changes brought about by this elongation can easily be seen in those figures in our joint paper which illustrate the development of the renal collar (Huntington and McClure, '20, figs. 8, 9, 10, and 11). Especially in figure 11 (H. and McC.) is clearly demonstrated the relation that exists between the elongated renal collar and the right sex vein (posterior cardinal).

An examination of a number of examples of *Type* AB in the cat has shown that the caudal extent of the periureteric venous ring varies in individual cases, extending caudad, as in figure 9, to the iliolumbar veins or, as in figure 10, only to a point somewhat craniad of this level. Differences in the caudal extent of the venous ring are dependent upon the extent to which either the right posterior cardinal or the right lumbar supracardinal vein may be involved in the ring's formation. When practically the entire right lumbar supracardinal vein persists, the venous ring extends caudad to the iliolumbar level (fig. 9). When only a portion of the right lumbar supracardinal vein persists, the caudal extent of the ring is determined by the level at which, cranial to the iliolumbar level, an anastomosis (*Pc.Sprc.Anast.*, fig. 1) is retained between the right posterior cardinal and the right supracardinal veins.

Type AB was found by Darrach in only three, or 0.5 per cent, of the adult cats examined. It was described for the cat by Gage in 1898; but, so far as we know, has been observed in man only by Wicke ('27)[6] and possibly by Melissinos in a doubtful case figured by him in 1911.

[6] This case described by Wicke is a most unusual one. In addition to the presence of a right periureteric venous ring (*Type* AB), the left common iliac vein passes ventral, instead of dorsal, to the right common iliac artery, indicating the persistence of a channel derived from the cardinal collateral vein (*C.C.* in fig. 1). See figure 63, plate XLVI.

3. Persistence of right posterior cardinal, right supracardinal, and left supracardinal veins. Right periureteric venous ring present, through which ureter passes

Type ABC (*A, B,* and *C* in fig. 1). Figure 11, cat (Darrach Series)

Figure 11 shows a case in which the vena cava posterior is represented in the lumbar region by three para-aortic channels, which enclose a right and a left venous ring. On the right side the ureter passes through the ring, establishing a typical periureteric venous ring. On the left side, however, the ureter extends between the kidney and the bladder without passing through the left venous ring. The relations which the ureters bear to the venous rings clearly indicate that the veins which form the rings have been derived from the right posterior cardinal and the right and the left supracardinal veins. The left para-aortic channel (left supracardinal vein) joins the right supracardinal, *dorsal* to the aorta, at a level somewhat caudal to that of the renal veins. This indicates that only a caudal section of the left lumbar supracardinal is concerned, and that by means of an intersupracardinal anastomosis, at a low level, it has retained a connection with the right supracardinal vein (*Int.Sprc.Anast.*, fig. 1).

So far as we can determine, the example illustrated by figure 11 is the only instance in which *Type* ABC has been described in the adult cat or in any other mammal.

4. Persistence of right posterior cardinal, right supracardinal, left supracardinal, and left posterior cardinal veins. Right and left periureteric venous rings present, through which ureters pass

Type ABCD (*A, B, C,* and *D* in fig. 1)

So far as we are aware, no instance of *Type* ABCD has ever been described, either for the adult cat or for man. The only case we know in which this type has been found to occur in an adult mammal is that described by Hochstetter for Erinaceus europaeus ('93, Taf. XXIII, fig. 24). Since *Type* ABCD uniformly occurs as a transitory stage in the develop-

ment of both cat and man, it is a potential condition present in the embryo, and, as a variant, might be carried into the adult stage.

5. Persistence of right posterior cardinal, right supracardinal, and left posterior cardinal veins. Right periureteric venous ring present, through which ureter passes

Type ABD (*A*, *B*, and *D* in fig. 1)

So far as we have determined, no example of *Type* ABD has thus far been observed, either in the adult cat or in any other mammal. As in the case of *Type* ABCD, *Type* ABD occurs as a transitory stage in the development of both cat and man and is therefore potential as a variant in the adult stage.

In this connection it is of interest to study a reconstruction of the veins in the lumbar region of a 21-mm. cat embryo. This reconstruction is instructive for the reason that it illustrates clearly the method by which venous transformations in the embryo lead to the formation of definite types of cavae.

On the right side of this embryo (fig. 12), there is present a periureteric venous ring, through which the ureter passes. The relation which the ureter bears to the ring clearly indicates that the veins which enclose the ring are the right posterior cardinal (*Pc., A*) and the right supracardinal veins (*Sprc., B*). On the left side of the embryo (fig. 13), the left posterior cardinal (*Pc., D*) has given up its connection with the supracardinal (*Sprc.*), *though only recently,* as is indicated, near the ureter (*Ur.*), by the narrow break in the left posterior cardinal vein (*Pc.*). We have here an instance in which *Type* ABD has recently been transformed into *Type* AB. Also, judging from the difference in size that exists between the right supracardinal (*Sprc.*, fig. 12) and the right posterior cardinal veins (*Pc.*), it seems probable, if development had proceeded further, that the right supracardinal (*Sprc.*) would have continued to atrophy and that the right posterior cardinal (*Pc.*) would alone have been retained in the adult to form a cava of *Type* A.

Another interesting feature of this embryo is the atrophied character of the left side of the renal collar (*Subc.Sprc. Anast.,* fig. 13), into which the left embryonic renal vein opens (*R.V.*). Ordinarily, the left side of the renal collar, with the left embryonic renal vein, breaks away from the supracardinal (*Sprc.*), and the left renal vein of the adult cat is thereby formed. The renal vein of the adult cat, formed in this manner by the left embryonic renal vein (*R.V.*), the left side of the renal collar (*Subc.Sprc.Anast.*), and the left subcardino-posterior cardinal anastomosis (*Subc.Pc.Anast.,* figs. 1 and 13), passes *ventral* to the aorta and receives the left sex vein (posterior cardinal and sex-vein tributaries) before joining the vena cava. However, if the left side of the renal collar with the embryonic renal vein (*R.V.*) should give up its connection with the subcardino-posterior cardinal anastomosis (*Subc.Pc.Anast.*) and retain its connection with the supracardinal vein (*Sprc.*), the left renal vein would then pass dorsal to the aorta before joining the caval vein. This latter condition is one not uncommonly found as a variant in the adult cat and in man, and is the normal condition found in the adult bat (Hochstetter, '93; Grosser, '01, and Butler, '27).

6. Persistence of right posterior cardinal and left supracardinal veins

Type AC (*A* and *C* in fig. 1). Figures 14, 15, and 16, cat (Darrach Series)

Of the two para-aortic axial veins shown in figures 14, 15, and 16, the vein of the right side is of posterior cardinal origin. This is evident from the relation which the right ureter bears to the vein. As in *Type* A (fig. 6), the right ureter on emerging from the kidney at first passes dorsal, and, farther caudad, ventral to the right cava.

The left para-aortic axial vein manifests considerable variation in the three examples shown. In one case (fig. 14) it extends only between the left iliac and the left iliolumbar veins, at which point it passes *dorsal* to the aorta to join the right caval vein (posterior cardinal). In another case (fig. 15) it forms a direct continuation of the left iliac vein and

joins the right cava (posterior cardinal) *dorsal* to the aorta, at a point somewhat caudal to the level of the renal veins. In the third case (fig. 16) the left para-aortic axial vein extends as a continuous vessel between the left iliolumbar and the left renal veins, and at about its caudal third receives the left spermatic vein, which in figures 14 and 15 opens into the left renal vein. In all three cases mentioned, the left ureter extends from the kidney to the bladder, without passing between the aorta and the left para-aortic axial vein. One may therefore interpret the left para-aortic axial vein in figures 14, 15, and 16 as due to the persistence in part (figs. 14 and 15), or in whole (fig. 16), of the left lumbar embryonic supracardinal vein. In figure 14 the caudal fifth, in figure 15 the caudal two-thirds, and in figure 16 the entire embryonic lumbar supracardinal vein have been retained. The connection, *dorsal* to the aorta, which the left para-aortic axial vein makes with the right cava in figures 14 and 15 is due to the persistence of anastomoses which, at different levels, exist in early stages between the left supracardinal and the right posterior cardinal veins (*Int.Sprc.Anast.* and *Pc.Sprc.Anast.*, fig. 1).

Type AC in the cat was first described by Gage in 1898. It was found in only four of the 605 cats examined by Darrach. We know of no instance in which *Type* AC has been found in man.

7. Persistence of right posterior cardinal, left supracardinal, and left posterior cardinal veins. Left periureteric venous ring present, through which ureter passes

Type ACD (*A*, *C*, and *D* in fig. 1). Figure 17, cat (Metcalf, '18)

Figure 17 is a photograph of *Type* ACD, described and figured by Metcalf as occurring in an adult cat. So far as we could determine, this variant marks the only instance on record in which *Type* ACD has been either observed or described as occurring in the adult cat or in any other mammal. Also, with the exception of Erinaceus europaeus, described by Hochstetter ('93), the case figured by Metcalf is the only

one on record in which a periureteric venous ring on the *left* side has been described as occurring in an adult mammal.

In the early stages of development a symmetrical arrangement of the main axial veins is a marked feature. One of the earliest changes which transposes the symmetrical embryonic plan to an asymmetrical one is the discontinuance of the left periureteric venous ring, through a break which occurs in the left posterior cardinal, near the point where the vein is crossed by the ureter (*Pc.* and *Ur.* in fig. 13). The early disappearance in ontogeny of the left periureteric venous ring undoubtedly accounts for the fact that this ring is rarely carried as a variant into the adult stage.

When compared with the composite ontogenetic plan of the veins (fig. 1), as typical an example of *Type* ACD as could exist is shown in figure 17. In the absence of an anastomosis, dorsal to the aorta, between the right and the left common iliac veins, all the blood from the right iliac veins is directed into the right posterior cardinal (right cava), about which the ureter winds. The right lumbar cava is composed only of the right posterior cardinal (*A*, fig. 1) and the right subcardino-posterior cardinal anastomosis (*Subc.Pc.Anast.*, fig. 1). The lateral portion of the renal collar (*Subc.Sprc.Anast.*) does not enter into the formation of the cava when the cava is formed by a posterior cardinal vein. A typical periureteric venous ring is present on the left side (fig. 17). The dorsal circumference of the ring is formed exclusively by the left supracardinal (*C* in figs. 1 and 17) and is of less importance functionally than the ventral circumference of the ring, which has been derived from the left posterior cardinal vein (*D* in fig. 1).

8. Persistence of right and left posterior cardinal veins

Type AD (*A* and *D* in fig. 1). Figures 18, 19, 20, 21, 22, 23, 24, cat (Darrach Series), and figure 25, man (Gladstone, '12)

Figures 18 to 24, inclusive, represent different variations of *Type* AD, in which two caval veins are present in the lumbar region, derived, respectively, from the right and the

left embryonic posterior cardinal veins. Their posterior cardinal origin is plainly manifested by the typical relation which the ureters bear to the veins (fig. 2). In *Type* AD there enters into the formation of the caval veins neither the lateral portions of the renal collar (*Subc.Sprc.Anast. Dext.* and *Sin.*, fig. 1) nor the supracardinal veins (*B* and *C*, fig. 1). However, in addition to the posterior cardinals (*A* and *D*, fig. 1), the right and the left subcardino-posterior cardinal anastomoses (*Subc.Pc.Anast.*, fig. 1) have also been retained. In figure 18 the right and the left caval veins are about subequal in size, while in figure 19 the vein of the right side is functionally the more important. There is considerable variation in the iliac region, in the connections between the caval veins of opposite sides. In some cases, as in figure 20, no connection whatever is present between these veins; in figure 21 only a slight one exists, through a right and a left fork of the caudal vein. Cases of incomplete fusion, in which arteries pass dorsal through foramina in the caval veins, recall early conditions in the embryo (figs. 3, 4, and 5), and are shown in figures 22 and 23; cases in which the fusion is complete in the iliac region between the veins of opposite sides are shown in figures 18, 19, 22, 23, and 24.

When either one or both of the caval veins have a posterior cardinal origin, it is important to note that the internal spermatic or sex veins, either single (fig. 19) or multiple (fig. 18), join the cava (posterior cardinal) somewhat caudal to the renal level. With the loss of the distal segment of the left posterior cardinal (figs. 18 to 24, inclusive), it is evident that the left spermatic vein would then, as under normal conditions, open into the left renal vein, and that a considerable portion of the left spermatic vein would be formed by the left posterior cardinal. This clearly demonstrates that the left spermatic vein of the adult is derived in part from the subcardinal and in part from the left posterior cardinal vein.

Type AD occurs with relative frequency in the adult cat. Darrach found it in twenty cases (3.3 per cent) of his series of 605 adults. In the cat it was first described by Hochstetter

('93), and later by Gage ('98), and McClure ('00). We know of but one instance on record in which it has been found in man. This is a case observed in an acardiac foetus by Gladstone (fig. 8, '12) and illustrated here by figure 25.

9. Persistence of right supracardinal vein. Normal vena cava posterior (inferior) in the cat and in man

Type B (*B* in fig. 1). Figures 26, cat (Darrach Series); 27, man (Flourens, '36); 28, man (Huntington Collection); 29, man (Huntington Collection); 30, man (Vesalius, 1543); 31, man (Froriep, '95), and 32, man (Huntington Collection)

In both cat and man the type of vena cava posterior represented by *Type* B is one in which, caudal to the renal veins, the cava is single and lies on the right side of the abdominal aorta. The right sex vein (V. spermatica interna) opens into the cava, slightly caudal to the level of the right renal vein, and the left sex vein into the left renal vein. On leaving the kidney, the right ureter extends directly to the bladder without passing between the aorta and the caval vein. *Type* B, in both cat and man, is due to the persistence in the adult of the right lumbar supracardinal vein (*Sprc. B*, fig. 1).

The sex veins in the cat are formed by the posterior cardinal and the subcardinal veins. An elongation of the right side of the renal collar (*Subc.Sprc.Anast.Dext.*, figs. 1 and 4), to the caudal border of which the posterior cardinal with its sex-vein tributary is attached, accounts for the entrance of the right sex vein into the cava, caudal to the level of the right renal vein. In the cat, after the loss of the left side of the renal collar (*Subc.Sprc.Anast.*, figs. 1 and 5), the left renal vein drains into the cava by the subcardino-posterior cardinal anastomosis (*Subc.Pc.Anast.*), to which the posterior cardinal with its sex-vein tributary is attached. In this manner may be explained the difference, which, in the cat, exists on opposite sides in the relations which the sex veins bear to the cava and to the left renal vein.

McClure and Butler ('25) have recently shown that in man the sex veins are derived exclusively from the subcardinal veins and that each subcardinal, near the intersubcardinal

anastomosis at the renal level (McC. and B., *Anast.Int.Subc.*, figs. 14 and 17), makes a cranial and a caudal connection with the axial veins. As the entrance of the right sex vein into the cava is in the adult usually single and caudal to that of the right renal vein, the more caudal of these two embryonic connections is the one usually retained. On the left side, the subcardinal (sex vein), by a cranial and a caudal connection, opens directly into the intersubcardinal anastomosis, which, after the loss of the left side of the renal collar (McC. and B., *Anast.Subc.Sprc.Sin.*, fig. 17), becomes the proximal portion of the left renal vein. The more caudal of the two embryonic connections which the left subcardinal vein makes with the intersubcardinal anastomosis is in the adult the one ordinarily retained.

Figures 26 to 32, inclusive, all represent instances in which the cava has been formed by the right supracardinal vein (*B*, fig. 1); they also illustrate certain variant or atypical conditions of the internal spermatic (V. spermatica interna) and the renal veins.

In figure 26 is shown an anastomosis between the sex veins of opposite sides, due to the persistence in the adult cat of an anastomosis present in the embryo, between the right and the left subcardinal veins (*Int.Subc.Anast.*, fig. 1). Such an anastomosis between sex veins has not yet been observed in adult man. This is undoubtedly due to the fact that corresponding intersubcardinal anastomoses are not found in the embryo of man (McClure and Butler, '25).

Figures 27 to 30, inclusive, of adult man, show interesting atypical conditions of the internal spermatic or sex veins. In figures 27, 28, and 29, the right sex vein communicates both with the cava and with the right renal vein; in figure 28, in addition to the double connection on the right side, the left sex vein presents also a double communication with the left renal vein. All these multiple connections which the sex veins present in adult man are easily explained on the basis of the persistence of the corresponding multiple connections found in the embryo of man (McClure and Butler, '25, figs.

14 and 17). Also, it is to be observed in figures 28 and 29 that the internal spermatic arteries show certain variant forms. It is of interest here to call attention to what is probably the earliest recorded observation of an atypical condition of the internal spermatic veins in adult man. In his "De Humani Corporis Fabrica," published in 1543, Vesalius shows a figure of the male urinogenital system of man in which the left internal spermatic vein presents a double communication with the cava and the left renal vein. The figure published by Vesalius is reproduced in our figure 30. In another figure, of the female urinogenital system, published also in 1543, Vesalius shows a single right and a single left sex vein opening, respectively, into the cava, caudal to the renal level, and into the left renal vein. As this is the normal condition in man, the multiple connection which Vesalius figured for the left sex vein must have been recognized by him as atypical in form.

The two remaining figures of this series, figures 31 and 32, illustrate two different forms of atypical renal veins.

Soon after emerging from the kidney, the left renal vein in figure 31 (*Fall.I.,* Froriep, '95) receives the left sex vein and then extends, caudad and mesad, *dorsal* to the aorta, to open into the cava, at the level of origin of the inferior mesenteric artery. The distinguishing features of this variant left renal vein are that it passes dorsal, instead of ventral, to the aorta and joins the cava somewhat caudal to the usual level of the renal veins. Except when a complete embryonic renal collar persists in the adult (fig. 59), instances in which the left renal vein passes *dorsal* to the aorta are associated with the absence of the left subcardino-posterior cardinal anastomosis (*Subc.Pc.Anast.,* fig. 1). In such cases, blood from the left posterior cardinal (*Pc.*) and the left embryonic renal vein (*R.V.*) is conveyed by the left side of the renal collar (*Subc. Sprc.Anast.*) to the left supracardinal (*Sprc.*), which may retain a connection with the right supracardinal (cava), dorsal to the aorta (*Int.Sprc.Anast.*), at any level in the lumbar region, between the kidneys and the common iliac veins. In

the case of the variant shown in figure 31, the cranial half of the left lumbar supracardinal forms the major portion of the left renal vein, which, caudal to the renal level and dorsal to the aorta, joins the caval vein. In this connection it is interesting to examine the conditions shown in figure 35. In this case, if the right renal vein were to give up its connection with the cava at the renal level, the right supracardinal, into which the right sex vein opens, would then function as a renal vein and, through its intersupracardinal anastomosis, would have the same relations to the sex vein, the aorta, and the vena cava, as does the left renal vein in figure 31. An essentially similar accessory left renal vein opening into the caval vein has been described by Watt ('20).

Another example of an atypical left renal vein is shown in figure 32. In this case, in addition to a right and a left renal vein that are typical in all respects in their relations to the cava and the sex vein, there is present an accessory left renal vein. On emerging from the left kidney, this accessory renal vein passes caudad and mesad to open into the cava, *ventral* to the aorta, at the point where the cava is joined by the common iliac veins. Since a typical renal vein is present on the left side, it is evident that this accessory renal vein cannot have been derived from the renal veins, which in the embryo open into the renal collar (McClure and Butler, '25). The only available embryonic pathways which could be retained in the formation of this accessory renal vein are the cardinal collateral (*C.C.*, figs. 1, 4, and 5) and the left subcardinal veins (*Subc.*, fig. 1). The manner in which its connection with the kidney came about is still problematical, although no doubt can exist about its cardinal collateral origin (*C.C.*, fig. 1) in the region where, ventral to the aorta, the accessory renal vein opens into the caval vein.

10. Persistence of right and left supracardinal veins

Type BC (*B* and *C* in fig. 1). Figures 33, 34, 35, man (Huntington Collection), 36, 37, 38, 39, 40, 41, 42, and 43, cat (Darrach Series)

The atypical venae cavae illustrated under *Type* BC present a variety of forms in which both the right and the left supracardinal veins of the embryo persist in the lumbar region, either wholly or only in part, in the adult cat and in man.

Figure 33 is perhaps a typical example of a case in which both lumbar supracardinal veins of the embryo have in their entirety been carried into the adult stage. In such cases the left cava (*C* in fig. 1) joins the cava of the right side (*B*), *ventral* to the aorta, and approximately at the level of the renal veins. As in all examples of *Type* BC, the ureters in figure 33 extend from the kidneys to the bladder without passing between the aorta and the caval (supracardinal) veins.

In figure 34, also of man, both lumbar supracardinals are present in their entirety and, as in figure 33, the left cava joins the cava of the right side, *ventral* to the aorta, at the level of the renal veins. A distinguishing feature of this variant, however, is that, functionally, the cava of the left side is the more important vessel of the two. Also, at about the middle of the lumbar region, the right cava receives a large spinal vein and, cranial to its union with this vein, the right cava is a vessel of much greater caliber than is that section of the cava which lies caudal to the spinal vein.

In figures 35 (man) and 36 (cat), both lumbar supracardinals are present in their entirety and, as in figures 33 and 34, the left cava joins the cava of the right side, *ventral* to the aorta, at the level of the renal veins. A distinguishing feature of these two variants is that, *dorsal* to the aorta, the right cava and the left again unite with each other, at a level somewhat caudal to that of the renal veins. This dorsal union between the two veins is due to the persistence of an intersupracardinal anastomosis (*Int.Sprc.Anast.*, figs. 1 and 3), which is present in the embryo, at different levels, between the supracardinals of opposite sides. Through the presence

of this intersupracardinal anastomosis, dorsal to the aorta, between the cavae of opposite sides, one can in the lumbar region (figs. 35 and 36) distinguish between a cranial and a caudal section of the right and the left caval veins. This distinction is one of considerable importance, as it enables us to interpret correctly those cases in which the left lumbar supracardinal is retained only in part, and not in its entirety, as in figures 33, 34, 35, and 36. If we compare figure 37 with figure 36, it is evident that the paired condition of the caval veins in figure 37 has been brought about by the persistence of the cranial section of the right and the left supracardinal veins as seen in figure 36. It is quite possible, however, that the unpaired caudal section of the lumbar cava in figure 37, as is usually the case in the cat, may have been formed by a fusion of both lumbar supracardinal veins (see figs. 2 and 3, in which an extensive anastomosis, dorsal to the aorta, is present between the supracardinal veins of opposite sides). Be this as it may, a fusion between the supracardinals cranially has not occurred, so that we have here a condition in which a paired cava is present, due to the persistence of a cranial section of the right and the left supracardinal veins as shown in figure 37.

In a similar manner the series of variants of *Type* BC, illustrated by figures 38 to 43, inclusive, can be interpreted as cases in which the entire right lumbar supracardinal and only a caudal section of the left supracardinal vein have been retained. In figures 38, 39, and 40, in which an anastomosis at the iliac level, between the iliac veins of opposite sides, has not been formed, it is seen that the left supracardinal joins the right supracardinal, *dorsal* to the aorta, at a level somewhat caudal to that of the renal veins. If we compare the elongated left common iliac vein in these three variants (figs. 38, 39, and 40) with the caudal section of the left supracardinal (left common iliac vein) in figure 36, we observe that, as in the latter, the elongated left common iliac must have been formed to a large extent by the left supracardinal vein, and that its point of union with the right supracardinal,

dorsal to the aorta, is determined in individual cases by the level at which an intersupracardinal anastomosis (*Int.Sprc. Anast.,* fig. 1) has been retained. In the composite diagram of the veins, figure 1, the line of development followed by the left cava in figures 38, 39, and 40 is along *C,* which, at different levels in individual cases, retains a connection, *dorsal* to the aorta, through an intersupracardinal anastomosis (*Int.Sprc.Anast.*) with the right supracardinal vein (*B*).

The variants shown of *Type* B in figures 41, 42, and 43 differ from those just described only in the presence of an additional anastomosis, dorsal to the aorta, at the iliac level (left common iliac vein in fig. 41), or, at the level of the iliolumbar veins (figs. 42 and 43), between the axial veins of opposite sides. If an anastomosis between the veins had not been established at the iliolumbar level in the variants shown in figures 42 and 43, there would have resulted an elongated left common iliac vein, similar to that seen in figure 40.

A double or paired vena cava posterior in the lumbar region (*Type* BC, fig. 1) is of relatively common occurrence as a variant both in the adult cat and in man, and is a type of cava normally met with in a number of mammalian forms. It has been found to occur normally in the adult of Phoca communis (von Baer, '35), Chalamydophorus truncatus (Hyrtl, '55), Phoca (spec.?), Halichoerus truncatus (Burow, '38); Phoca vitulina, Delphinium delphis, Dasypus setosus, Dasypus novemcinctus, Manis gigantea, Erinaceus europaeus, Pteropus edulis, Echidna setosa (Hochstetter, '93); Orycteropus (Beddard, '09), and probably in the elephant (Huntington and McClure).

In mammals other than man, a double posterior vena cava has been described occurring as a variant in the dog by Kerkring (1670); in the cat by Gage ('98), McClure ('00), Darrach ('07), Smallwood ('06), and Metcalf ('18); in Hylobates by Keith ('96); and in Didelphys virginiana by McClure ('03 and '06).

Among the early anatomists who have described or figured a double vena cava inferior as occurring in adult man are:

Blasius (1674), Petsche (1736), Wilde (1750), Timmermann (1750), Lobstein (1760), Meckel (1773), Pohl (1773), and Morgagni (1779). In more recent times it has been described or figured as occurring in adult man by: Alten ('13), Antonelli ('82), Augier ('09), Barkow ('66), Brin ('97), Broca ('52), Carrière ('14), Cousin ('98), Cruveilhier ('35), Farmer ('97), Fawcett ('98), Flesch ('76), Gérard ('03, '06, and '14), Gerlach ('85), Givens ('12), Gorron ('00), Gruber ('80 and '81), Hahn ('96), Huber, cited by Serres ('60), Johnston ('13), Kadyi ('81), Kollmann ('93), Lagneau ('53), Lauber ('01), Looney ('22), Le Gendre ('60), Leudet ('53), Lobstein ('30), Lucas ('16), Martin ('52), Meckel ('22), Nicolai ('86), Oberndorfer ('03), Osler ('78), Otto ('24), Pangratz ('94), Parsons ('96), Patten ('09), Roth, cited by Serres ('60), Quain ('44), Rischbieth ('14), Serres ('60), Shepherd ('90), Soemmerring (1800), Stark ('34), Stieda ('93), Tichomirow ('93 and '98), Wallin ('20), Walsham ('81), Walter ('84), Watt ('20), Waterston ('13), Wilbrand ('42), Zaaijer ('73), Zagorsky ('22), Zander ('92), Zander and Stieda ('92), Zimmermann, cited by Serres, and Zumstein ('95). Although from an historical standpoint the above list is in no sense complete, nevertheless it indicates the relative frequency with which this variant (*Type* BC) has been observed in adult man.

Variant conditions of the vena cava inferior in which the posterior cardinal veins are concerned are exceedingly rare in adult man (*Types* A, AB, ABC, ABD, AC, ACD, AD, BCD, BD, CD, and D, fig. 1). See table on page 48. McClure and Butler ('25) have explained why this is the case, on the supposition that, since the posterior cardinal veins lose their identity and disappear early in ontogeny, their chance of being carried into the adult stage is correspondingly slight. Be this as it may, this view coincides exactly with the conditions found in adult man, in which variant forms of the vena cava, as thus far observed, consist principally of cases in which only the supracardinals are involved (*B* and *C*, fig. 1).

11. Persistence of right and left supracardinal, and left posterior cardinal veins. Periureteric venous ring present on left side, through which ureter passes

Type BCD (*B, C,* and *D* in fig. 1)

So far as we are aware, no example of *Type* BCD has thus far been observed either in the adult cat or in man. An interesting example of *Type* BCD, however, in which the right and the left supracardinal and the left posterior cardinal veins are involved, has been found to occur as a transitory ontogenetic stage in a 17-mm. embryo of the cat. Here, in figures 44 and 45, of the right and the left sides, respectively, of a reconstruction of the veins, both supracardinals (*Sprc.Dext.* and *Sin.*) are still functional as embryonic caval veins and the left supracardinal (*Sprc.Sin.,* fig. 45), with the left posterior cardinal vein (*Pc.Sin.,* fig. 45), forms a complete periureteric venous ring through which the ureter passes. It is evident that, potentially, the condition presented in this embryo may be carried into the adult stage. The chances are, however, that if development had proceeded to the adult stage, only the right supracardinal (*Sprc.Dext.,* fig. 44) and the left posterior cardinal veins (*Pc.Sin.,* fig. 45) would have been retained to establish a cava of *Type* BD, as shown in figures 46 and 47. The large size of the right supracardinal (*Sprc.Dext.,* fig. 44) and the left posterior cardinal veins (*Pc. Sin.,* fig. 45) leads one to infer that blood would still be directed from the iliac veins into these two vessels, at the expense of the smaller left supracardinal (*Sprc.Sin.,* fig. 45) and the left side of the renal collar (*Subc.Sprc.Anast.,* fig. 45). Although *Type* BCD has not thus far been observed in the adult cat, it is of importance to recognize the fact that it is found in the embryo and is therefore potential in the adult.

12. Persistence of right supracardinal and left posterior cardinal veins

Type BD (*B* and *D* in fig. 1). Figures 46 and 47, cat (Darrach Series)

Type BD represents a case of double vena cava posterior. It differs from *Type* BC (fig. 33) in that the left cava has

been derived from the left posterior cardinal (*D* in fig. 1), instead of from the left supracardinal vein (*C* in fig. 1). This is evident from the fact that the left ureter passes dorsal to the left cava and emerges between the aorta and the caval vein. On the right side the cava is normal, having been derived from the right supracardinal vein (*B* in fig. 1), as is indicated by the relation which the ureter bears to the right caval vein.

Darrach found only two examples of *Type* BD in his series of 605 adult cats, and both of these are shown here in figures 46 and 47. So far as we know, *Type* BD has not hitherto been described for cat or for man.

In figure 46, the right supracardinal and the left posterior cardinal veins are of equal caliber. As no communication exists between the iliac veins of opposite sides, each cava starts as a direct continuation of the common iliac vein. In figure 47, the right supracardinal forms the larger of the two caval veins. It starts from the iliac confluence and ascends on the right side of the aorta, passing ventral to a large aberrant renal artery. The left posterior cardinal, the smaller of the two caval veins, starts from the left iliac vein and ascends at first dorsal and then ventral to the ureter, to join the right cava, ventral to the aorta, approximately at the level of the renal veins. In both instances the sex veins, except for their duplicity on the left side, present normal relations to the caval veins.

Figures 48 and 49 represent, respectively, the right and the left sides of a reconstruction of the veins in a 23-mm. cat embryo. As shown in the figures, the right supracardinal (*Sprc.Dext.*, fig. 48) and the left posterior cardinal veins (*Pc.Sin.*, fig. 49) are the only vessels (cavae) concerned in returning blood to the intersubcardinal anastomosis (*Int. Subc.Anast.*, fig. 49) from the iliac veins. The type of vena cava represented at this stage of development is, therefore, *Type* BD—a condition which may have been only transitory in character or, if development had proceeded, might have been carried into the adult stage. This 23-mm. cat embryo

is therefore another instance of the potential character of the embryonic venous system in relation to the establishment of atypical forms of caval veins.

13. Persistence of left supracardinal vein

Type C (*C* in fig. 1). Figures 50 and 51, cat (Darrach Series)

The vena cava posterior represented by *Type* C is a mirror picture of *Type* B. In *Type* C the vena cava is a single vessel, situated on the left side of the abdominal aorta, derived from the left embryonic supracardinal vein (*C* in fig. 1). As in all cases where the cava has been formed by a supracardinal, the ureter in *Type* C lies entirely free of the caval vein. The left sex vein opens into the cava caudal to the renal level, and the right sex vein opens usually into the right renal (fig. 50), though occasionally into the caval vein (fig. 51).

Figure 52 represents the left side of a reconstruction of the veins in a 45-mm. cat embryo. It is an example of a case in which the left supracardinal vein (*Sprc.*, fig. 52) has persisted to form the caval vein (*Type* C). It is of special interest as evidence that the opening of the left sex vein into the cava, caudal to the level of the renal veins (*R.V.*), has been brought about by an elongation of the lateral portion of the renal collar (*Subc.Sprc.Anast.*), to the caudal border of which the posterior cardinal vein (*Pc*), with its sex-vein tributary (*S.V.*), is attached.

The presence of a left-sided vena cava posterior of *Type* C is of relatively frequent occurrence in the adult cat. It was found by Darrach ('07) in twenty-one cases (3.4 per cent) of his series of 605 adults. Also, it has been observed in the cat by Gage ('98), McClure ('00), and Hunt ('19); and in Didelphys virginiana by McClure ('03 and '06). Among the earlier anatomists a left vena cava inferior (*Type* C) was observed in man by Wrisberg in 1778 and by Morgagni in 1779. In more recent times it has been described as occurring in man by Berthel ('01), Cameron ('11), Fränkel ('10), Georg ('06), Gérard ('08), Gladstone ('12), Grimsdale ('94), Gruber

('65), Harrison ('39), McWhinnie ('40), Oberndorfer ('03), Paterson ('01), Quain ('44), Waring ('94), and Zumstein ('95).

Type C and *Type* BC are the variants of the vena cava inferior which occur most frequently in adult man.

14. Persistence of left supracardinal and left posterior cardinal veins. Periureteric venous ring present on left side, through which ureter passes

Type CD (*C* and *D* in fig. 1)

No instance has thus far been described, either in the adult cat or in man, in which the left supracardinal and the left posterior cardinal veins have alone persisted to form a periureteric venous ring on the left side. *Type* CD is a mirror picture of *Type* AB (fig. 9), with the venous ring on the left instead of the right side. Two cases have been described in mammals in which a periureteric venous ring was present on the left side—but in combination, however, either with a supracardinal or with a supracardinal and a posterior cardinal on the right side. The two cases referred to were described, respectively, by Hochstetter ('93) in Erinaceus europaeus (*Type* ABCD) and by Metcalf ('18) in the cat (*Type* BCD). (See figure 17 of the present monograph, in which Metcalf's figure has been reproduced.) While the presence of a periureteric venous ring on the left side is of rare occurrence in the adult cat, it is always present in the embryo (fig. 45), and is therefore potential for the adult.

15. Persistence of left posterior cardinal vein

Type D (*D* in fig. 1). Figure 53, cat (Darrach Series)

Type D is a mirror picture of *Type* A, in which the cava is formed by the right posterior cardinal vein. *Type* D represents a case in which, caudal to the renal level, the cava is single, and lies on the left side of the abdominal aorta, with the left ureter passing between the aorta and the caval vein (fig. 53). The position which the left ureter bears to the cava indicates that the latter was derived from the left posterior cardinal vein.

Type D was found by Darrach ('07) in twenty-one (3.4 per cent) of his series of 605 adult cats. Also, it has been described in the cat by Hunt ('19). With the exception of a very doubtful case mentioned by Lawrence in 1897, *Type* D has not thus far been found in man.

16. Persistence of embryonic supracardinal system of veins to form a continuous system of vessels in the lumbar and the thoracic regions, by means of which blood from the iliac region can be conveyed directly to the heart, without passing through the liver

(*B* and *C*, and supracardinal (azygos), *dext.* and *sin.*, in fig. 1). Figures 54, man (fig. 5, Fawcett, '98); 55, man (fig. 1, Lauber, '01); 56, man (fig. 3, Kollmann, '93); 57, man (Hochstetter, '93), and 58, cat (Princeton Morphological Museum, no. 1040)

This type of variant has been designated by previous writers as 'absence of vena cava posterior' and as 'persistence of posterior cardinal veins.' From the standpoint of its development, this variant in both cat and man is an example of the persistence of the supracardinal complex of veins which, in the embryo, extend as continuous channels through the lumbar and the thoracic regions. In both cat and man, the bilaterally symmetrical supracardinal veins of the embryo subsequently anastomose freely with each other, dorsal to the aorta, in both the thoracic and the lumbar regions, though in the latter, in man less freely than in the cat (McClure and Butler, '25). From this complex are typically derived in its lumbar segment (postrenal) the normal adult cava and, in the thoracic region, the azygos vein (Huntington and McClure, '20). When the supracardinal veins persist in the adult as a continuous system throughout the lumbar and the thoracic regions, blood from the iliac veins can be returned to the heart without passing through the liver. (See *B* and *C* and supracardinal (azygos) in fig. 1.)

As thus far observed in the adult cat and in man, all cases of atypical vena cava posterior, which result from the persistence in the lumbar and the thoracic regions of a continuous

supracardinal system of veins, may be classed under two general groups:

Group I includes those cases in which blood from the iliac veins is returned to the heart by the supracardinal system of veins, as well as by the usual and normal route through the liver.

Group II includes those cases in which all blood from the iliac veins is returned to the heart by the supracardinal system of veins, and in which the usual and normal route through the liver, via the prerenal division of vena cava, has not been established.

Figures 54 and 55 are excellent examples of cases which fall within group I. In each instance blood from the iliac veins has, in returning to the heart, a choice of two routes: one through the liver, by the prerenal division of the vena cava, and the other, by the supracardinal system of veins, which open into the superior vena cava and into the left innominate vein. As shown in figure 54 (fig. 5, Fawcett, '98), the embryonic condition of a bilaterally symmetrical and continuous system of supracardinal veins in the lumbar and the thoracic regions has actually been carried into the adult stage. In this case the supracardinals are represented in the lumbar region by the right and the left caval veins (*Type* BC), and, continuous with the latter in the thoracic region, by the azygos veins. In addition to the supracardinals, there is present the prerenal division of the vena cava inferior. This vessel communicates with the supracardinals at the renal level, and from this point extends craniad through the liver to open into the right auricle. The left azygos has been designated by Fawcett (fig. 54) as a derivative of the left cardinal vein. This view we now know to be incorrect. With the exception of the proximal end of the right and the left azygos veins, which are derived from the posterior cardinal veins (*Pc.1* in fig. 1), the continuous channel in the lumbar and the thoracic regions on each side of the body has been derived from the supracardinal system of veins. In figure 55 (fig. 1, Lauber, '01) is shown a similar condition, less dia-

grammatically represented than in figure 54. In this instance the right and the left caval veins in the lumbar region (supracardinals, *Type* BC), incorrectly designated by Lauber as posterior cardinal veins, are directly continuous in the thoracic region with a single azygos vein, which, in the middle thoracic region, swings, dorsal to the aorta, from the left to the right side, and opens into the vena cava superior. This single azygos vein shows indications of having been formed by a fusion, dorsal to the aorta, between the supracardinal veins of opposite sides. In addition to the supracardinal line as a pathway for the return of blood to the heart from the iliac region, there is present also a typical vena cava inferior (*V.cava inf.*, fig. 55), which joins the supracardinal system at the renal level and, after passing through the liver, opens into the right auricle.

We now pass to a consideration of those cases which fall within group II. These are illustrated by figures 56, 57, and 58. Here the supracardinal system of veins has persisted in the adult and constitutes the sole and only direct main pathway by which blood is returned to the heart from the region of the body drained by the iliac veins. In all variants which fall under group II, the intrahepatic venous circulation, formed by the advehent portal and the revehent hepatic channels, is entirely independent of the systemic veins (supracardinals). In figure 58 are shown the portal vein entering the liver and the vena hepatica communis emerging from the liver and extending to the right auricle. An ontogenetic interpretation of this form of variant is relatively simple. During the course of normal development, a connection is established between the hepatic circulation and the systemic veins (right subcardinal), at a point designated the *hepato-subcardinal junction* (McClure, '06), so that blood is directed from the systemic veins to the heart through the liver. (See Huntington and McClure, '20, figs. 1 and 2.) Should this connection not be established, the only available pathway from the posterior region of the body to the heart would be by the supracardinal system of veins. If that portion of the vena

cava inferior which extends between the hepatic veins (Vv. hepaticae) and the renal level had not developed, an essentially similar condition would then prevail, as is shown in figures 56, 57, and 58. An examination of figures 55 to 58, inclusive, shows variations in the extent to which the originally bilaterally symmetrical supracardinal veins of the embryo may be carried into the adult. In figure 54 the supracardinal system has been carried into the adult in essentially its embryonic form; in figures 55 and 56 the right and the left supracardinals are present in the lumbar region, while in the thoracic region only the caudal half of the left and the cranial half of the right supracardinal have been retained. In figure 57 the right supracardinal along its entire extent has been carried into the adult stage. In this instance a considerable portion of the left thoracic supracardinal has entered into the formation of the left renal vein.

The occurrence of variants of this character in the adult cat and in man serves as a complete confirmation of the presence in the embryo of a supracardinal system of veins, which constitutes a morphological unity, distinct from that of the embryonic posterior cardinal veins.

An examination of the literature shows that an atypical condition of the veins, which has been designated by previous writers either as 'absence of inferior vena cava' or as 'persistence of posterior cardinal veins,' has been described a number of times as occurring in man and, in a few cases, in other mammals, as follows:

1. In man, by Abernathy (1793), Barkow ('66), Benöhr ('04), Bujalsky ('29), Carpentier et Bertaux ('88), Cruveilhier ('71), Dorsch ('58), Dwight ('01), Goettsch ('20), Gurlt ('19), Herholdt ('32), Hochstetter ('93), Horner ('17), Hyrtl ('39), Kaestner ('00), Lauber ('01), Klob ('59), Kollmann ('93), Lawrence ('19), Martin ('62), McWhinnie ('40), Moser ('48), Neuberger ('13), Osler ('78), Otto ('24), Paulus ('42), Quain ('44), Revell ('03), Tourneux et Wertheimer ('82), Virchow ('61), Wertheimer ('80), Winslow (1775), and Wistar (1827).

2. In mammals other than man, it has been described in the cat, by Huntington and McClure, Pearl ('08), and Hunt ('19); in the dog, by Ponsot ('56), Körösy ('10), and Cooper ('20); in the deer (Cervus tarandus), by Barkow ('66); and in the guinea-pig (Cavia) by Phisalix ('98).

17. Persistence of renal collar (circumaortic venous ring at level of renal veins)

See renal collar in figure 1. Figure 59, man (Huntington Collection)

The renal collar is a term used by Huntington and McClure to designate an extensive embryonic venous ring, which, like a collar, encircles the aorta, at the level of the renal veins. In the embryo of the cat (fig. 1) the renal collar is formed ventrally by the intersubcardinal anastomosis (*Int.Subc. Anast.*) and the right and the left subcardino-posterior cardinal anastomoses (*Subc.Pc.Anast.*); laterally, by the right and the left subcardino-supracardinal anastomoses (*Subc. Sprc.Anast.*); and dorsally, by the intersupracardinal anastomosis (*Int.Sprc.Anast.*) between the supracardinal veins. The renal collar serves in the embryo as a common meeting place for all main axial venous channels and is a region toward which blood is directed from the systemic veins to the heart, by way of the liver, through the prerenal division of the vena cava posterior (*P.Subc.* and *P.Hep.*, fig. 1). In transforming from the embryonic to the adult condition, the renal collar early loses its ring-like character, and only a portion of it is retained in the adult cat, where it enters into the formation of the left renal vein (*Subc.Pc. Anast.Sin*) and of a small segment of the vena cava posterior (*Subc.Sprc.Dext.*, fig. 1). For further details the reader is referred to the article by Huntington and McClure ('20), on "The development of the veins in the domestic cat."

In figure 59 we have represented an instance in which the renal collar, in essentially its embryonic condition, has persisted in adult man. In two other cases observed in man, one shown in figure 35 and the other figured by Oberndorfer ('03), the aorta is encircled by a venous ring, but not in so typical

a form at the renal level as that shown in figure 59. In all three cases, however, the portion of the venous ring which lies dorsal to the aorta has resulted from the persistence of an intersupracardinal anastomosis (*Int.Sprc.Anast.*, fig. 1), between the supracardinal veins.

The three cases above mentioned represent the only instances on record in which a venous ring, encircling the aorta at or near the renal level, has been carried into the adult.

18. Persistence of subaortic plexus (embryonic cardinal collateral veins)

Marsupial Type of vena cava posterior. (See *C.C.* in fig. 1 and in figs. 4 and 5, for cardinal collateral veins). Figure 61, cat (Darrach Series); 62, cat (Princeton Morphological Museum, no. 603), and 63, man (Wicke, '27)

In most placental mammals the iliac veins unite to form the vena cava posterior *dorsal* to the aorta and to the iliac arteries; and, between the iliac and the renal veins, the cava lies free of the ureter and to the *right* of the aorta. This is the type of cava (*Type* B) which is ordinarily met with in placental mammals. On the other hand, in most of the Australian marsupials, the iliac veins unite to form the vena cava posterior *ventral* to the aorta and to the iliac arteries; and, between the iliac and the renal veins, the cava lies free of the ureters and *ventral* to the aorta. A comparison of figure 26 of the cat with figure 60 of an Australian marsupial (Petrogale) clearly illustrates the difference between the typical vena cava posterior of a placental mammal (*Type* B) and that of a marsupial.

In both cat (Huntington and McClure, '20) and man (McClure and Butler, '25) the umbilical arteries of the embryo are at one time encircled by venous rings, the ventral circumference of which is formed by a vein, designated cardinal collateral vein (*C.C.* in fig. 1). The term *cardinal collateral* was first applied by McClure ('06), in marsupials, to certain embryonic veins, distinct from the posterior cardinals. In the lumbar region these veins lie ventrolateral and ventral to the aorta, and *ventral* to the umbilical arteries. In the adult

marsupial, these give rise in the lumbar region to the vena cava posterior. The cardinal collaterals in the embryo cat (*C.C.*, figs. 1, 4, and 5), as in marsupials, lie ventral and ventrolateral to the aorta and extend throughout the lumbar region, between the iliac and the renal veins (*C.C.* in figs. 4 and 5). While typical circumumbilical venous rings are present in the human embryo, the cardinal collateral system of veins in the lumbar region between the iliac and the renal veins is not so prominently developed in the embryo of man as in that of the cat (McClure and Butler, '25). Since cardinal collateral channels are normally met with during the course of ontogeny, they like other normal embryonic veins are potential in the adult stage. As a matter of fact, we know of four such instances, two in the cat and two in man.

Figure 61 represents a case in which the embryonic cardinal collateral (*C.C.* in fig. 1), in conjunction with the right and the left posterior cardinal veins (*A* and *D* in fig. 1), has persisted in an adult cat. The vena cava posterior represented by this variant is one which we may regard as being a combination of *Type* AD and of the so-called *Marsupial Type*. If we compare figures 18 and 19, each of which is a good example of *Type* AD, with figure 61, we observe that in one case the iliac veins unite *dorsal,* and in the other, *ventral* to the aorta, to form the vena cava posterior. Also, if we compare the variant in figure 61 with the marsupial cava in figure 60, we observe that, with exception of the relation which the right ureter bears to the right caval vein, the conditions are in each case identical, as regards the relations between the aorta and the right caval vein.

In the preceding pages we have seen that there are fifteen ways in which the lumbar cava may be formed and carried into the adult stage, due to the persistence of one or more of the embryonic right and left supracardinal and the embryonic right and left posterior cardinal veins. It is therefore evident we may rightly expect that the cardinal collateral vein (*C.C.* in fig. 1) might persist in the adult cat in conjunction with any one of these fifteen possibilities or *Types* of caval

veins. Such being the case, if the right supracardinal (*B* in fig. 1) had persisted in the variant (fig. 61) in place of the right posterior cardinal, the relations of the cava to the ureter and the aorta would in the variant and the marsupial have been exactly the same.

Another example of a case in which the cardinal collateral vein of the embryo (*C.C.*) has been retained in an adult cat is shown in figure 62. Also, a case identical to this has been described and figured by Johnston ('13, p. 235), as occurring in adult man. While these two cases are not so typical as that shown in figure 61, nevertheless they represent instances in which a vein, extending ventral to the aorta between the left iliac and the caval veins (right supracardinal, *Type* B), has been derived from the ventral portion of the embryonic circumumbilical venous rings (*C.C.* in fig. 1). An analysis of figure 1 clearly shows the possibilities which exist as regards the persistence of such a ventral venous channel as that shown in figure 62. Its origin from the cardinal collateral vein of the embryo is the only plausible explanation of its presence in the adult cat and in man.

Another example of the *Marsupial Type* has more recently been described and figured for adult man by Wicke ('27).[7] In this case (fig. 63), a periureteric venous ring, through which the ureter passes, is present on the right side (*Type* AB); in addition to this venous ring, however, there is present a persistent cardinal collateral channel, represented by the left common iliac vein (*V.il.c.s.*), which passes *ventral* to the right common iliac artery (*A.il.c.d.*), to become directly continuous with the right posterior cardinal vein (*V.cv.r.*).

[7] Über einen Fall von Inselbildung im Bereiche der hinteren Hohlvene (Vena cava posterior) und Durchtritt des rechten Harnleiters beim Erwachsenen. Zeitsch. für Anat. und Entwicklungsgesch., Bd. 84, S. 524–533.

This paper did not appear until after Doctor Huntington's death. Also, since 1927, two other important papers have appeared dealing with atypical conditions of the vena cava posterior:

Maxwell, E. V., and Erwin, G. S. 1928. Four cases of anomalous inferior vena cava with an explanation of their development. Jour. Anat., vol. 62, pp. 184–197.

Kadletz, M. 1928. Über eine Missbildung im Bereich der Vena cava caudalis beim Hunde. Zeitsch. für Anat. und Entwicklungsgesch., Bd. 88, S. 385–396.

The ventral position occupied by the left common iliac vein clearly indicates that it has been derived from the ventral portion of the embryonic circumumbilical venous rings (*C.C.* in fig. 1). Furthermore, the presence of the anastomosis between the right and the left common iliac veins (*V.cm.cd.*) indicates the persistence of a complete embryonic circumumbilical venous ring on the right side, as is shown in the composite diagram of the veins (*C.C.*, fig. 1).

It is of interest here to note that during the course of this investigation our prediction that a cava of the *Marsupial Type* would be found in both cat and man has been completely fulfilled.

The table that follows shows the relative frequency with which the different types of vena cava posterior have been observed to occur in the adult cat and in man. The data for the cat include those cases observed by Darrach in his series of 605 adults, as well as those recorded in the literature by other investigators. The data for man include cases which have been recorded in the literature available to us. While the table is in no sense to be regarded as complete, it nevertheless brings out many interesting points regarding the relative distribution of the different types of venae cavae as thus far determined by us in the cat and in man.

VII. TABLE SHOWING RELATIVE FREQUENCY WITH WHICH THE DIFFERENT *TYPES* OF VENA CAVA POSTERIOR HAVE BEEN OBSERVED TO OCCUR IN THE ADULT CAT AND IN MAN

TYPE	CAT	MAN
A	24	4
AB	4	1
ABC	1	0
ABCD	0	0
ABD	0	0
AC	5	0
ACD	1	0
AD	23	1
B	Normal vena cava	Normal vena cava
BC	14	71
BCD	0	0
BD	2	0
C	24	17
CD	0	0
D	22	1(?)
Persistent supracardinal system	3	33
Persistent renal collar	0	3
Marsupial Type	2	2

VIII. GENERAL CONCLUSIONS

In the preceding pages we have demonstrated that all atypical conditions of the vena cava posterior (inferior) found in the adult cat and in man can be interpreted and explained on the basis of the modifications of a common ontogenetic plan of the veins. An analysis of the composite diagram (fig. 1) of the embryonic veins shows that seventeen (17) predictable forms of atypical venae cavae occur. In conclusion, we may state that every atypical condition of the vena cava thus far observed in the cat and in man falls within one of the seventeen predictable *Types* of caval veins.

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X. ABBREVIATIONS

A., lumbar division of right posterior cardinal vein (V. cardinalis posterior dextra)

Adr., adrenal vein (V. adrenalis, suprarenalis)

A.Om., omphalomesenteric artery (A. omphalomesenterica)

Ao., aorta

B., lumbar division of right supracardinal vein (V. supracardinalis dextra)

C., lumbar division of left supracardinal vein (V. supracardinalis sinistra)

C.C., ventral portion of circumumbilical venous ring in figure 1 (V. cardinalis collateralis). Also V. cardinalis collateralis in figures 4 and 5

D., lumbar division of left posterior cardinal vein (V. cardinalis posterior sinistra)

D.C., duct of Cuvier (V. cardinalis communis)

E.Il.V., external iliac vein (V. iliaca externa)

I.Il.V., internal iliac vein (V. iliaca interna)

Int.Sprc.Anast., intersupracardinal anastomosis. Anastomosis, dorsal to aorta, between supracardinal veins

Int.Subc.Anast., intersubcardinal anastomosis. Anastomosis, ventral to aorta, between subcardinal veins

K., permanent kidney (metanephros)

L.Innom.V., left innominate vein (V. anonyma sinistra)

P.Hep., pars hepatica of vena cava posterior

P.Subc., pars subcardinalis of vena cava posterior

Pc., posterior cardinal vein (V. cardinalis posterior)

Pc.1., thoracic division of posterior cardinal vein forming cranial end of azygos vein

Pc.2.Dext., lumbar division of right posterior cardinal vein entering into formation of right periureteric venous ring

Pc.2.Sin., lumbar division of left posterior cardinal vein entering into formation of left periureteric venous ring

Pc.Sprc.Anast., posterior cardinal-supracardinal anastomosis (anastomosis between posterior cardinal and supracardinal veins)

R.V., embryonic renal vein

Sprc., supracardinal vein (V. supracardinalis)

Sprc.Dext.(*P.Sprc.*), right supracardinal vein (pars supracardinalis of vena cava posterior)

Subc., subcardinal vein (V. subcardinalis)

Subc.Pc.Anast., subcardino-posterior cardinal anastomosis. Anastomosis between subcardinal and posterior cardinal veins

Subc.Sprc.Anast., subcardino-supracardinal anastomosis. Anastomosis between subcardinal and supracardinal veins

S.V., sex vein (V. spermatica interna)

Ur., ureter

V.Sper.Int., sex vein (V. spermatica interna)

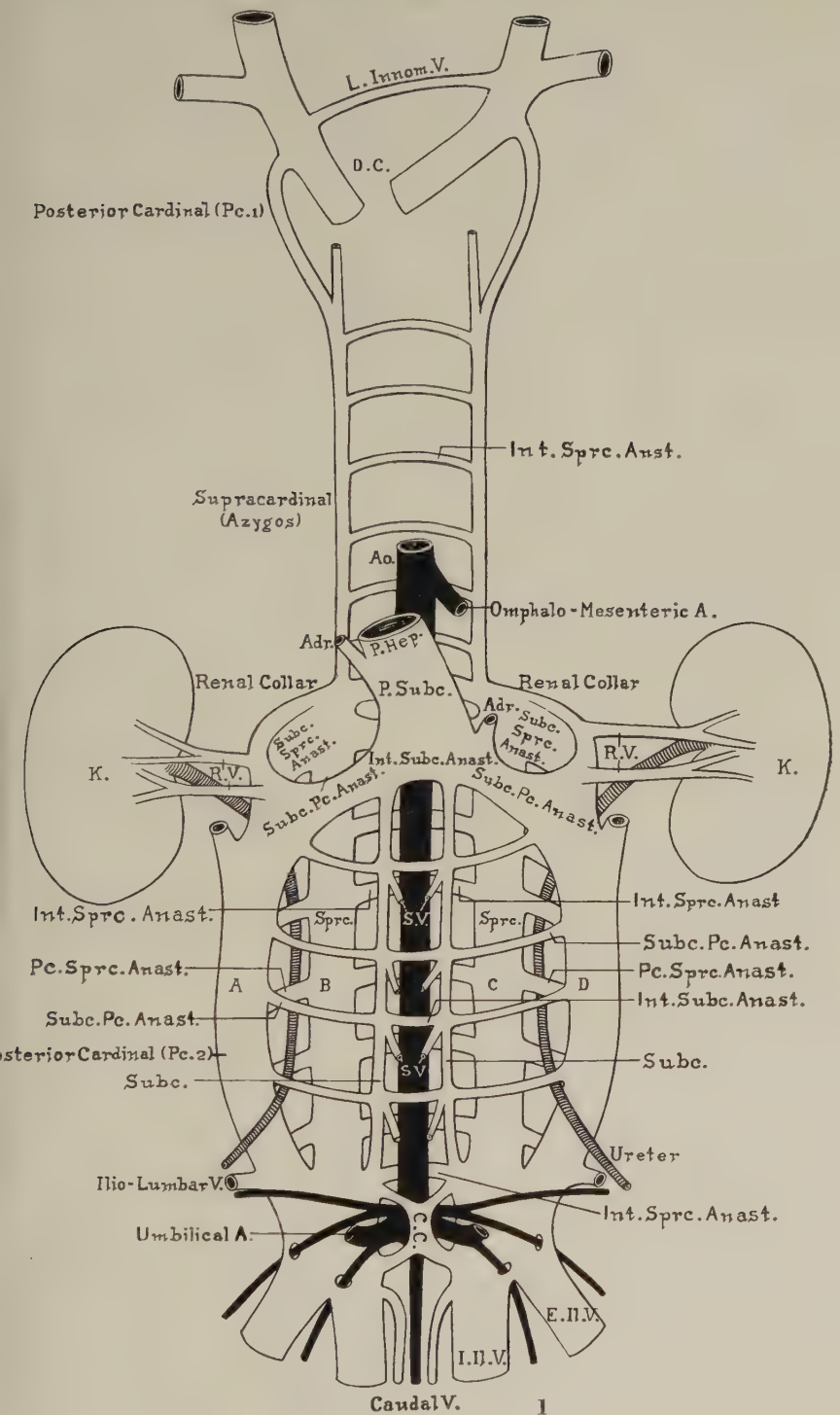
XI. PLATES

PLATE I

EXPLANATION OF FIGURE[9]

1 Composite diagram of embryonic veins of the domestic cat (Felis domestica), based on a detailed study of the development of the veins.

[9] The figures in this memoir were drawn by Mr. M. Petersen, of the College of Physicians and Surgeons of Columbia University.


L. Innom. V.
D.C.
Posterior Cardinal (Pc.1)
Int. Sprc. Anst.
Supracardinal (Azygos)
Ao.
Omphalo-Mesenteric A.
Adr.
P. Hep.
Renal Collar
P. Subc.
Renal Collar
Adr.
Subc. Sprc. Anast.
Subc. Sprc. Anast.
K.
R.V.
Int. Subc. Anast.
R.V.
K.
Subc. Pc. Anast.
Subc. Pc. Anast.
Int. Sprc. Anast.
Sprc.
S.V.
Sprc.
Int. Sprc. Anast
Subc. Pc. Anast.
Pc. Sprc. Anast.
A
B
C
D
Pc. Sprc. Anast.
Int. Subc. Anast.
Subc. Pc. Anast.
osterior Cardinal (Pc.2)
Subc.
S.V.
Subc.
Ureter
Ilio-Lumbar V.
Int. Sprc. Anast.
Umbilical A.
C.C.
E.Il.V.
I.Il.V.
Caudal V.

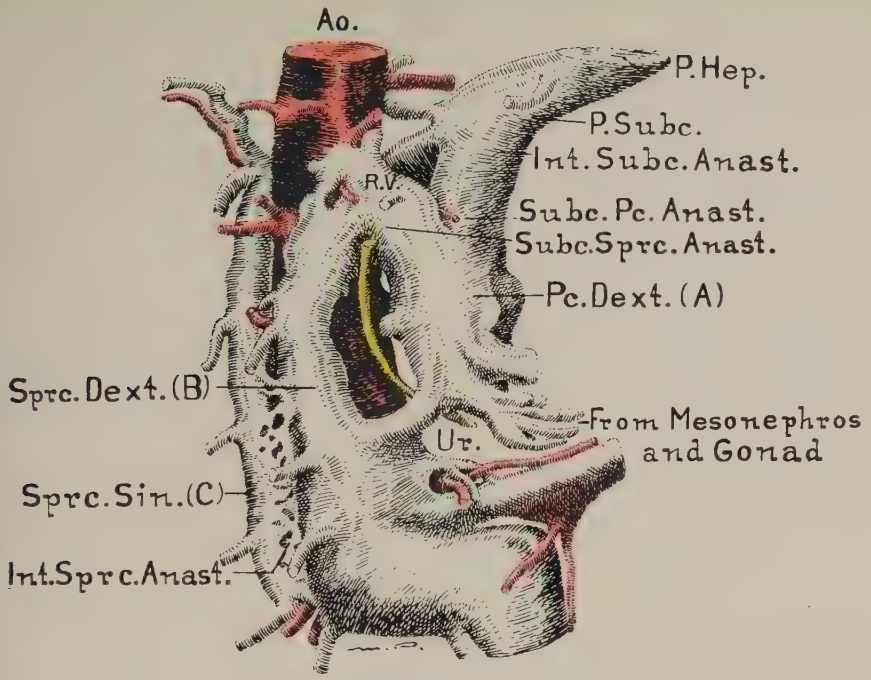
1

PLATE II

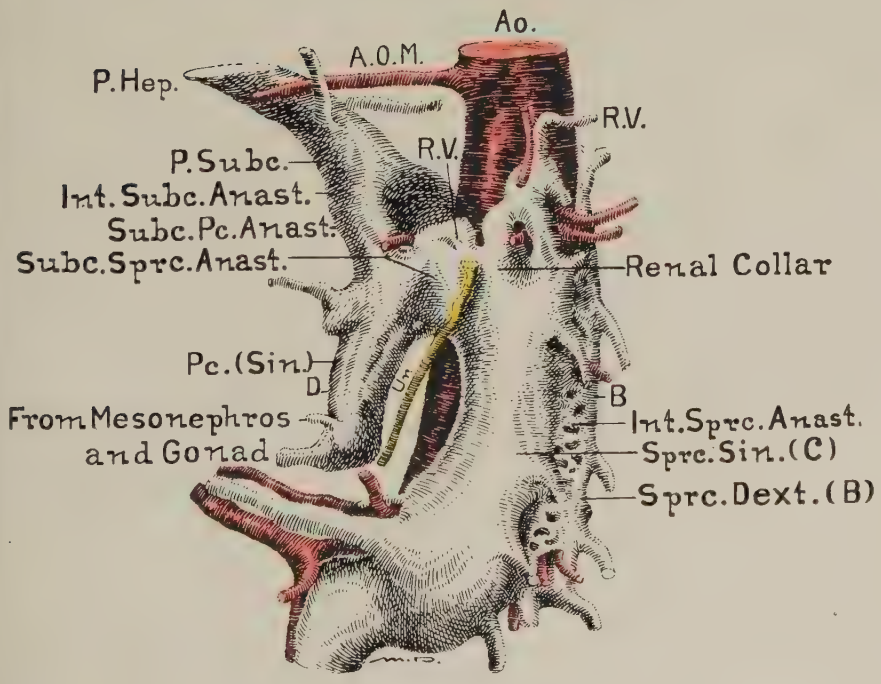
EXPLANATION OF FIGURES

2 Reconstruction (after Born) of veins in the lumbar region of a 16-mm. cat embryo. Lateral aspect, *right* side, showing the right posterior cardinal (*A*), the right supracardinal (*B*), the left supracardinal (*C*), the right periureteric venous ring, through which the ureter (*Ur.*) passes, and the left side of the renal collar (*Subc.Sprc.Anast.*). Princeton Embryological Collection, no. 15.

3 Reconstruction (after Born) of veins in the lumbar region of a 16-mm. cat embryo. Lateral aspect, *left* side, showing the left posterior cardinal (*D*), which has lost its caudal connection with the left supracardinal (*C*), so that a periureteric venous ring is not present on the left side. The left posterior cardinal (*D*), with its subcardinal sex-gland tributaries, forms the left V. spermatica interna in the adult. The right posterior cardinal (*A*, fig. 2) similarly forms the vena spermatica of the right side. Note that the posterior cardinals (*A* and *D*), on either side of the body, joins the caudal border of the lateral portion of the renal collar (*Subc.Sprc.Anast.*) and that the right and the left supracardinals (*B* and *C*) anastomose with each other, dorsal to the aorta, by intersupracardinal anastomoses (*Int.Sprc.Anast.*). The vena cava in this embryo is still in a transitory state of development and at present is represented by a cava of *Type* ABC. Princeton Embryological Collection, no. 15.


Ao.
P.Hep.
P.Subc.
Int.Subc.Anast.
R.V.
Subc.Pc.Anast.
Subc.Sprc.Anast.
Pc.Dext.(A)
Sprc.Dext.(B)
From Mesonephros
and Gonad
Ur.
Sprc.Sin.(C)
Int.Sprc.Anast.

2


Ao.
A.O.M.
P.Hep.
R.V.
R.V.
P.Subc.
Int.Subc.Anast.
Subc.Pc.Anast.
Subc.Sprc.Anast.
Renal Collar
Pc.(Sin.)
D.
B
From Mesonephros
and Gonad
Int.Sprc.Anast.
Sprc.Sin.(C)
Sprc.Dext.(B)

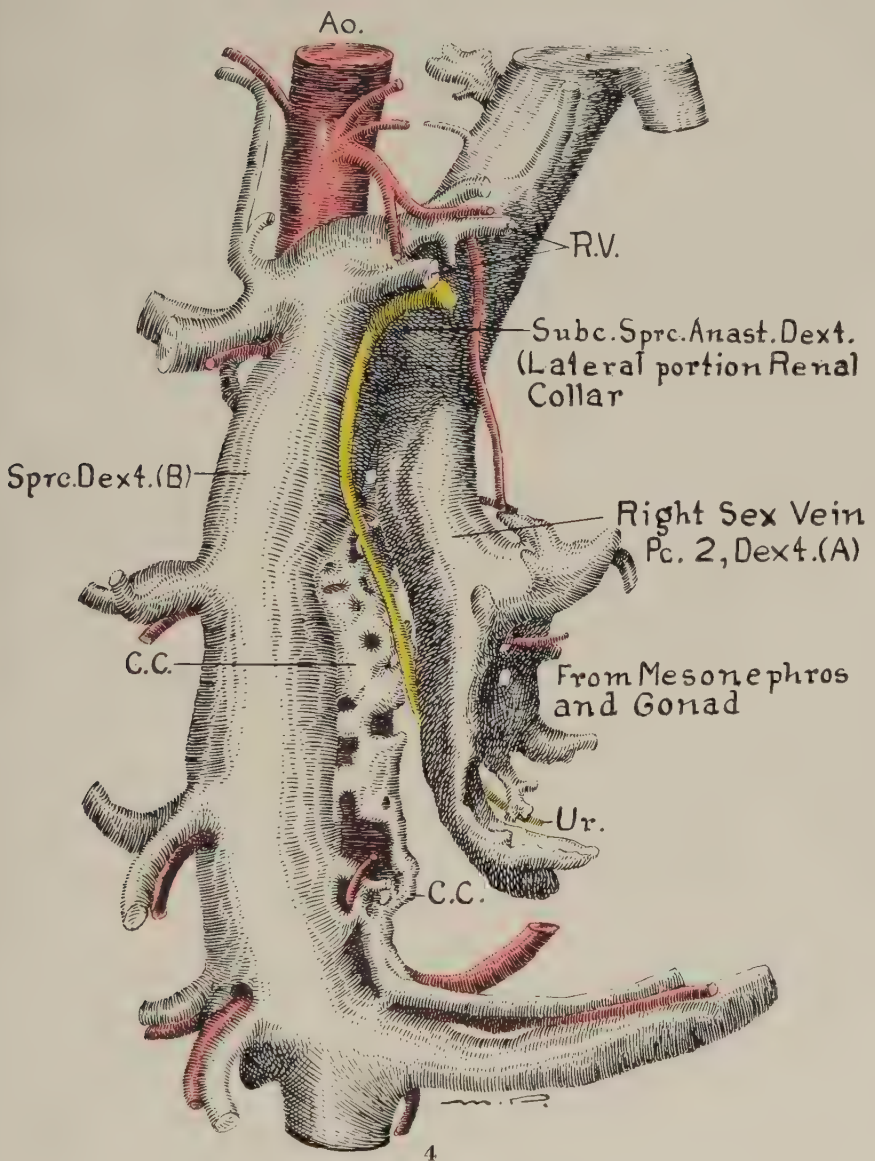
3

PLATE III

EXPLANATION OF FIGURE

4 Reconstruction (after Born) of veins in the lumbar region of a 29-mm. cat embryo. Lateral aspect, *right* side, showing the cardinal collateral system of veins (*C.C.*), which extend, ventral and ventrolateral to the aorta, between the iliac and renal levels. Note the right vena spermatica interna (*Pc.2.Dext.*) and its relations to the ureter (*Ur.*) and to the lateral portion of the renal collar (*Subc.Sprc.Anast.Dext.*). Coll. of P. and S. Embryological Collection, no. 575.[10]

[10] College of Physicians and Surgeons of Columbia University.


Ao.
R.V.
Subc.Sprc.Anast.Dext.
(Lateral portion Renal
Collar
Sprc.Dext.(B)
Right Sex Vein
Pc. 2, Dext.(A)
C.C.
From Mesonephros
and Gonad
Ur.
C.C.

4

PLATE IV

EXPLANATION OF FIGURE

5 Reconstruction (after Born) of veins in the lumbar region of a 29-mm. cat embryo. Lateral aspect, *left* side, showing cardinal collateral system of veins (*C.C.*), and complete atrophy of left side of renal collar (*Subc.Sprc. Anast.*). Note that, with the loss of the left side of the renal collar, blood in the left supracardinal (*C*) is directed into the right supracardinal, through the dorsal intersupracardinal anastomoses, present between the right and the left supracardinal veins. At the present stage of development the cava in the lumbar region appears to be formed through a fusion of both supracardinal veins. Coll. of P. and S. Embryological Collection, no. 575.

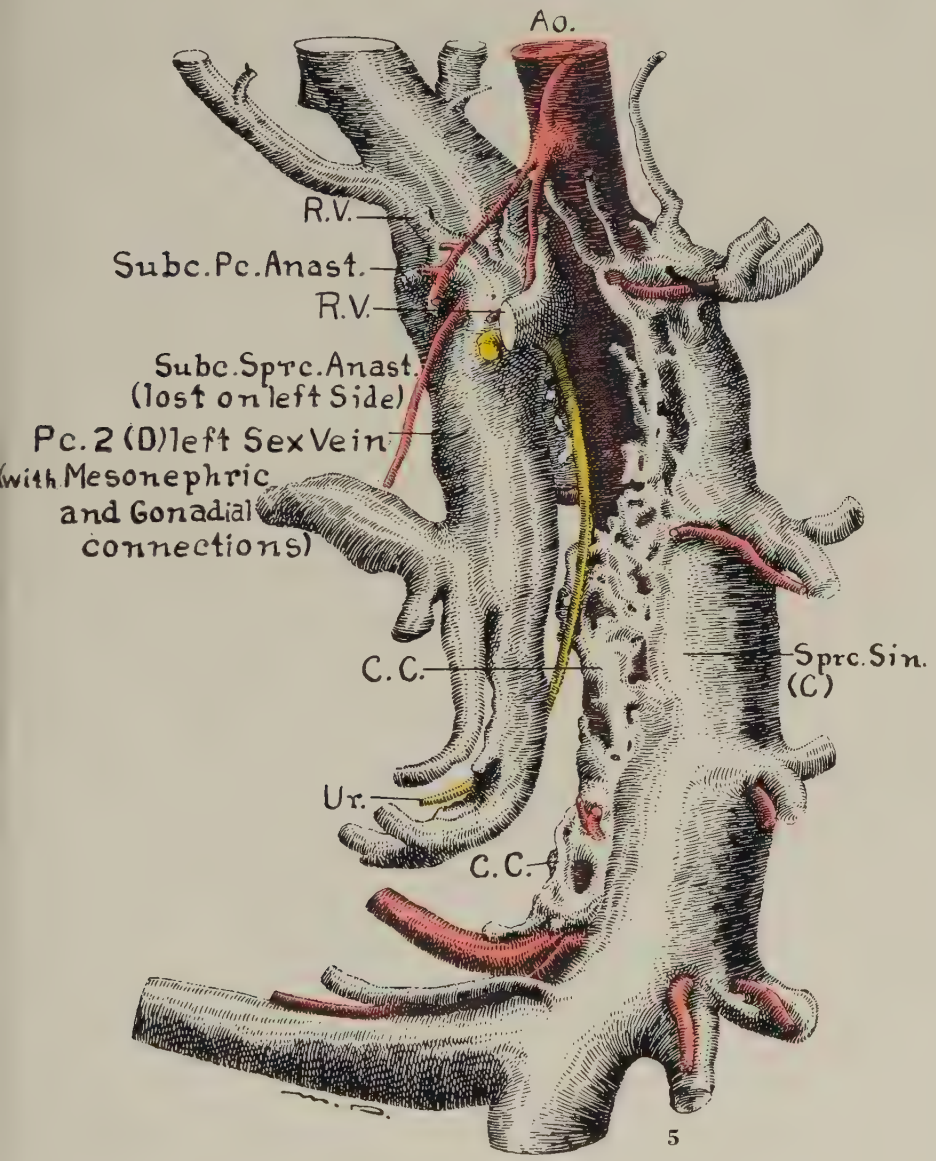
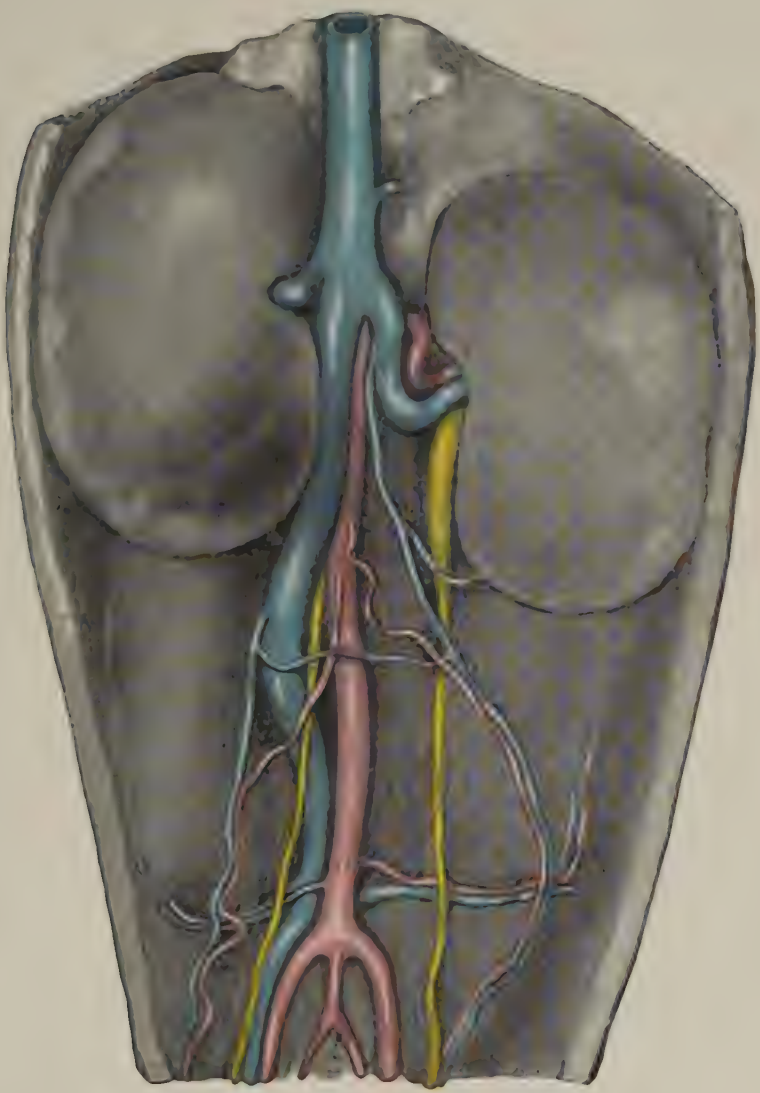

Ao.
R.V.
Subc.Pc.Anast.
R.V.
Subc.Sprc.Anast.
(lost on left Side)
Pc.2 (D) left Sex Vein
(with Mesonephric
and Gonadial
connections)
C.C.
Sprc.Sin.
(C)
Ur.
C.C.
5

PLATE V

EXPLANATION OF FIGURE

6 Adult cat (Darrach Series), *Type* A. Persistence of right posterior cardinal vein.



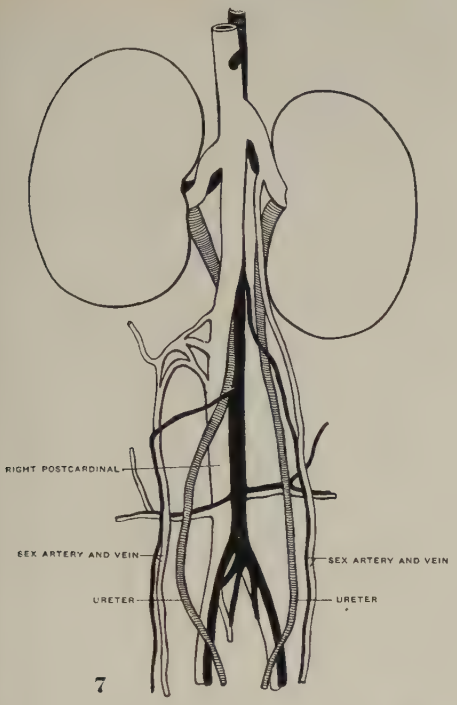
6

PLATE VI

EXPLANATION OF FIGURES

7 Adult cat (Darrach Series), *Type* A. Persistence of right posterior cardinal vein.

8 Adult man (Gladstone, fig. 1, '12), *Type* A. Persistence of right posterior cardinal vein.


RIGHT POSTCARDINAL
SEX ARTERY AND VEIN
SEX ARTERY AND VEIN
URETER
URETER

7

8

PLATE VII

EXPLANATION OF FIGURE

9 Adult cat (Darrach Series), *Type* AB. Persistence of right posterior cardinal and right supracardinal veins. Periureteric venous ring, through which ureter passes, present on right side.

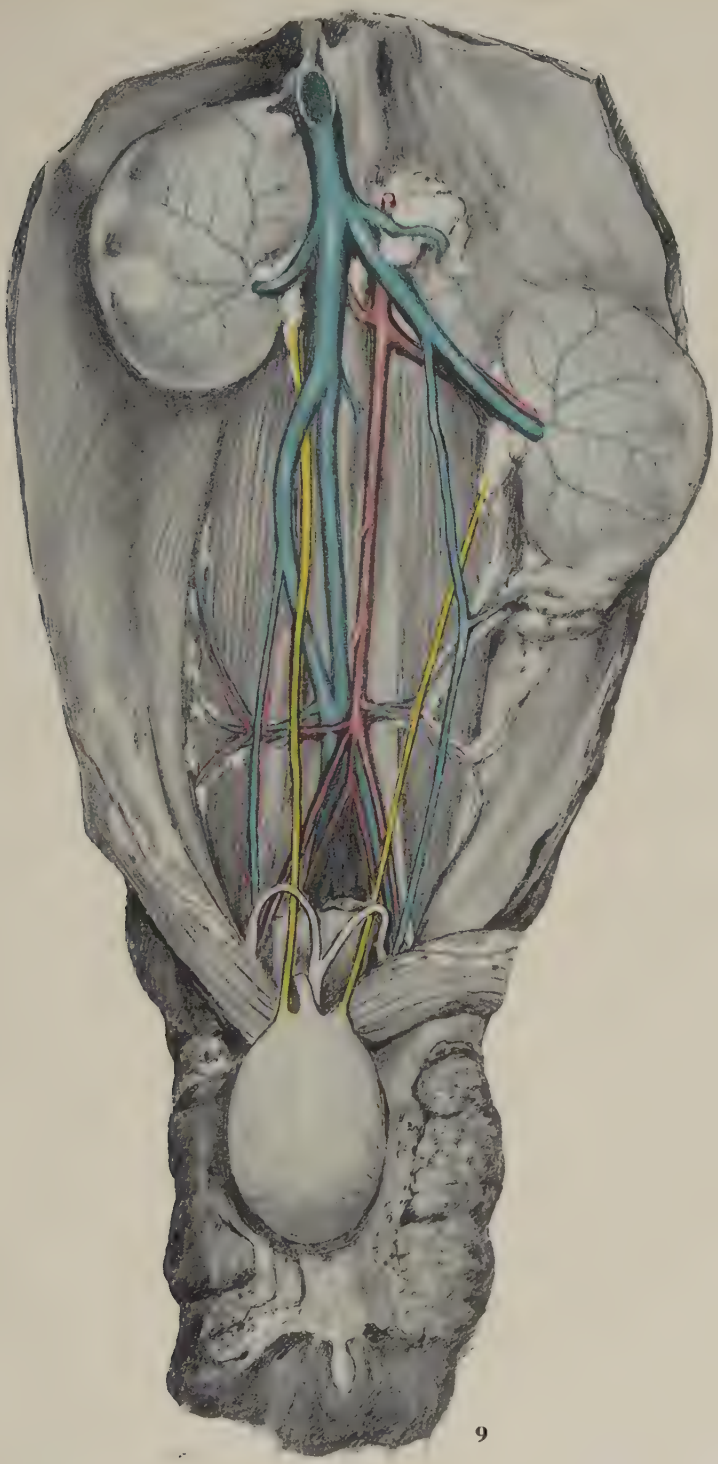


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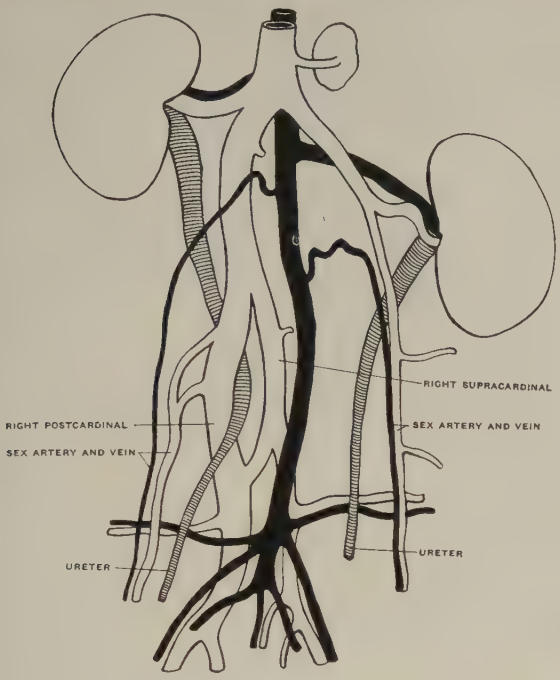
PLATE VIII

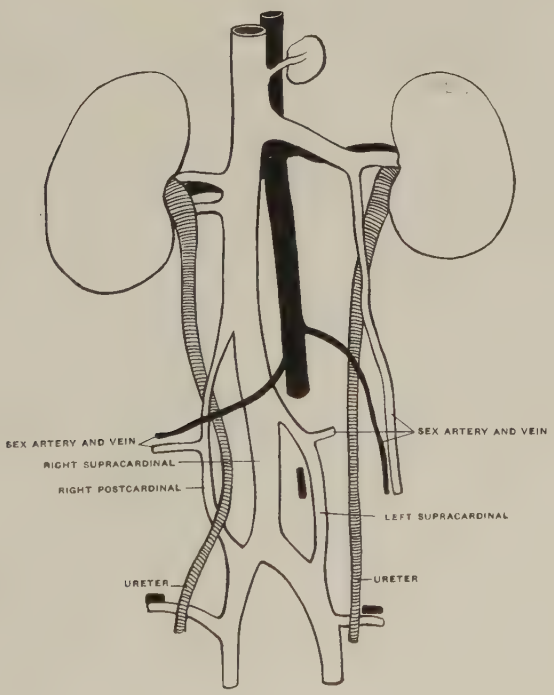
EXPLANATION OF FIGURES

10 Adult cat (Darrach Series), *Type* AB. Persistence of right posterior cardinal and right supracardinal veins. Periureteric venous ring, through which ureter passes, present on right side.

11 Adult cat (Darrach Series), *Type* ABC. Persistence of right posterior cardinal, right supracardinal, and left supracardinal veins. Periureteric venous ring, through which ureter passes, present on right side.


RIGHT SUPRACARDINAL
RIGHT POSTCARDINAL
SEX ARTERY AND VEIN
SEX ARTERY AND VEIN
URETER
URETER

10


SEX ARTERY AND VEIN
SEX ARTERY AND VEIN
RIGHT SUPRACARDINAL
RIGHT POSTCARDINAL
LEFT SUPRACARDINAL
URETER
URETER

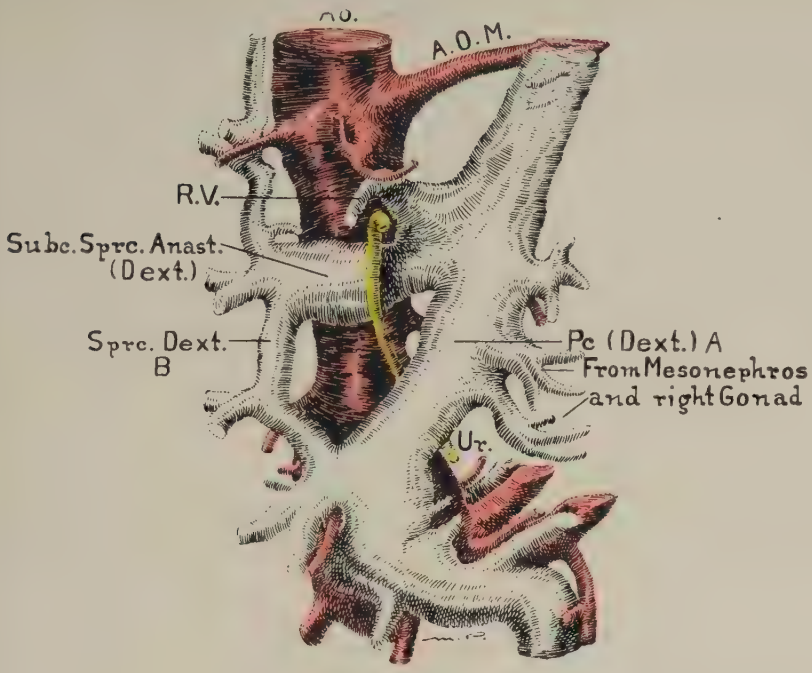
11

PLATE IX

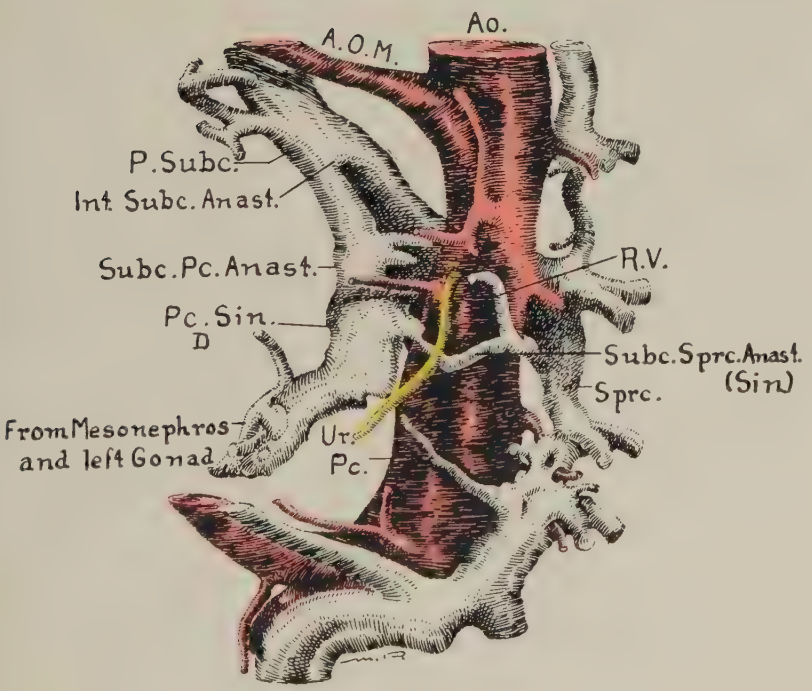
EXPLANATION OF FIGURES

12 Reconstruction (after Born) of veins in the lumbar region of a 21-mm. cat embryo. Lateral aspect, *right* side. Periureteric venous ring, through which ureter passes, present on *right* side. Coll. of P. and S. Embryological Collection, no. 242.

13 Reconstruction (after Born) of veins in the lumbar region of a 21-mm. cat embryo. Lateral aspect, *left* side. Coll. of P. and S. Embryological Collection, no. 242.


Ao.
A.O.M.
R.V.
Subc. Sprc. Anast.
(Dext.)
Sprc. Dext.
B
Pc (Dext.) A
From Mesonephros
and right Gonad
Ur.

12


A.O.M.
Ao.
P. Subc.
Int. Subc. Anast.
Subc. Pc. Anast.
R.V.
Pc. Sin.
D
Subc. Sprc. Anast.
(Sin)
Sprc.
From Mesonephros
and left Gonad
Ur.
Pc.

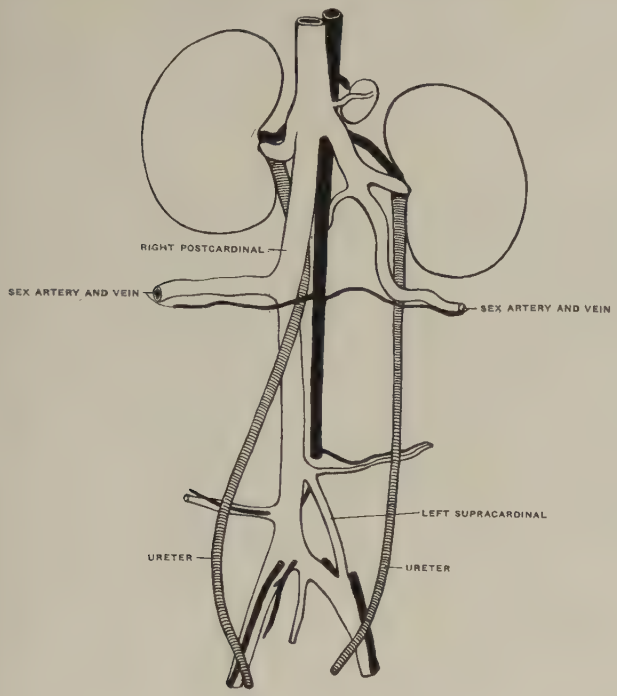
13

PLATE X

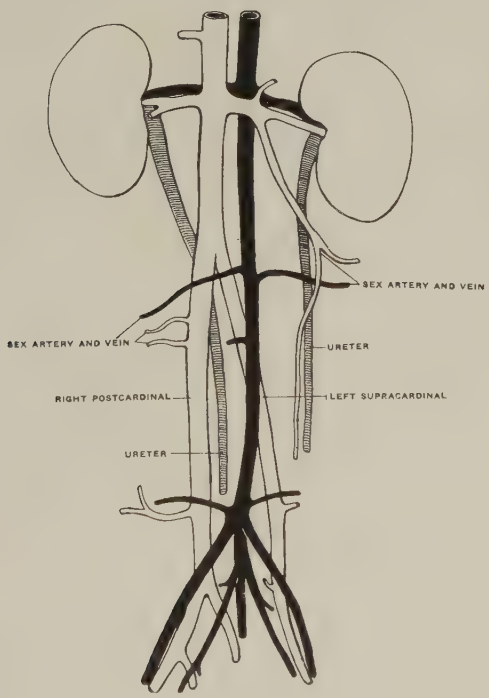
EXPLANATION OF FIGURES

14 Adult cat (Darrach Series), *Type* AC. Persistence of right posterior cardinal and left supracardinal veins.

15 Adult cat (Darrach Series), *Type* AC. Persistence of right posterior cardinal and left supracardinal veins.


RIGHT POSTCARDINAL
SEX ARTERY AND VEIN
SEX ARTERY AND VEIN
LEFT SUPRACARDINAL
URETER
URETER

14

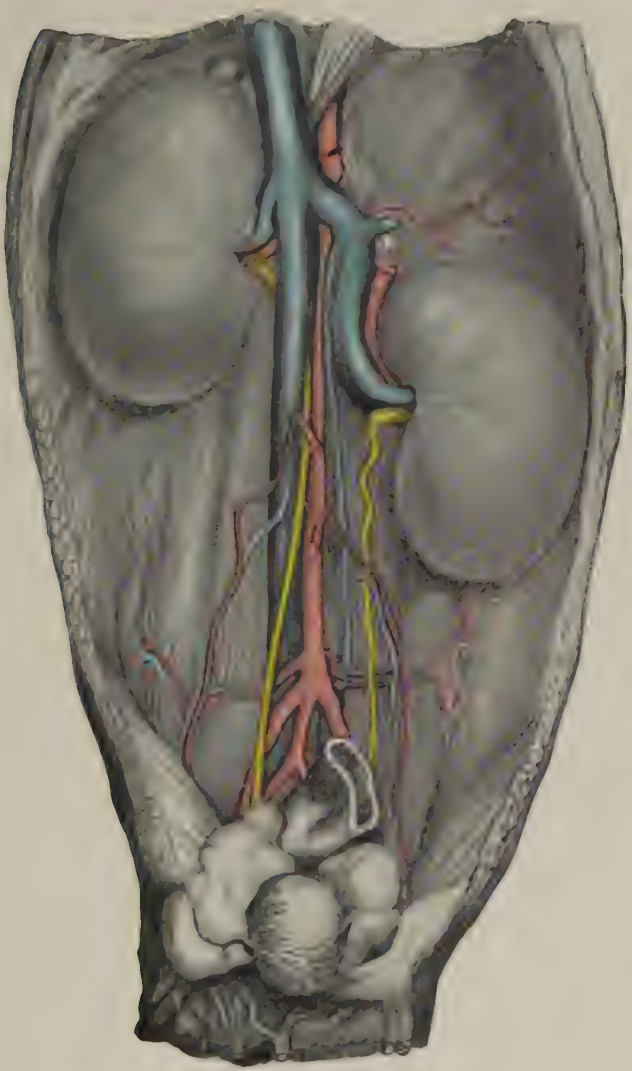

SEX ARTERY AND VEIN
SEX ARTERY AND VEIN
URETER
RIGHT POSTCARDINAL
LEFT SUPRACARDINAL
URETER

15

PLATE XI

EXPLANATION OF FIGURE

16 Adult cat (Darrach Series), *Type* AC. Persistence of right posterior cardinal and left supracardinal veins.

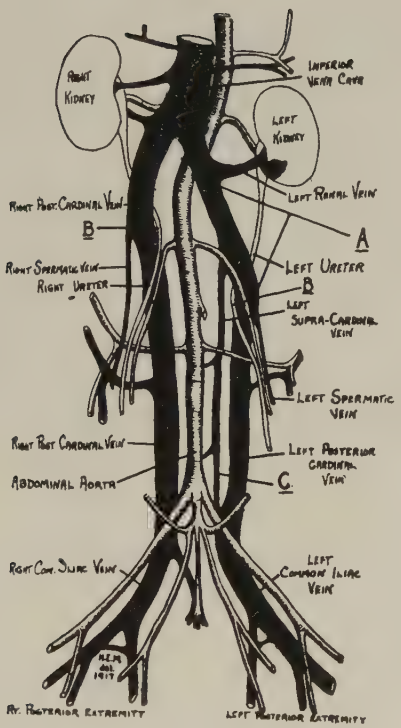


16

PLATE XII

EXPLANATION OF FIGURE

17 Adult cat (from Metcalf, fig. 1, '18), *Type* ACD. Persistence of right posterior cardinal, left supracardinal, and left posterior cardinal veins. Perirureteric venous ring, through which ureter passes, present on left side. Legends by Metcalf.

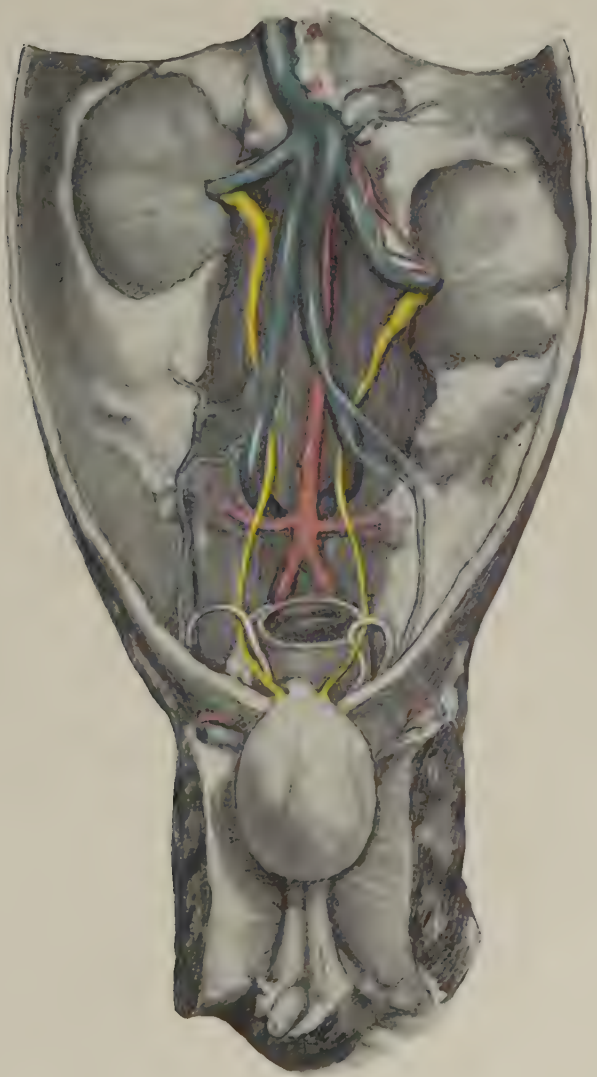

Right Kidney
Inferior Vena Cava
Left Kidney
Left Renal Vein
Right Post. Cardinal Vein
B
A
Left Ureter
Right Spermatic Vein
Right Ureter
B
Left Supra-Cardinal Vein
Left Spermatic Vein
Right Post Cardinal Vein
Left Posterior Cardinal Vein
Abdominal Aorta
C
Right Com. Iliac Vein
Left Common Iliac Vein
Rt. Posterior Extremity
Left Posterior Extremity

17

PLATE XIII

EXPLANATION OF FIGURE

18 Adult cat (Darrach Series), *Type* AD. Persistence of right and left posterior cardinal veins. Ureters pass between aorta and caval veins.



18

PLATE XIV

EXPLANATION OF FIGURE

19 Adult cat (Darrach Series), *Type* AD. Persistence of right and left posterior cardinal veins. Ureters pass between aorta and caval veins.

19

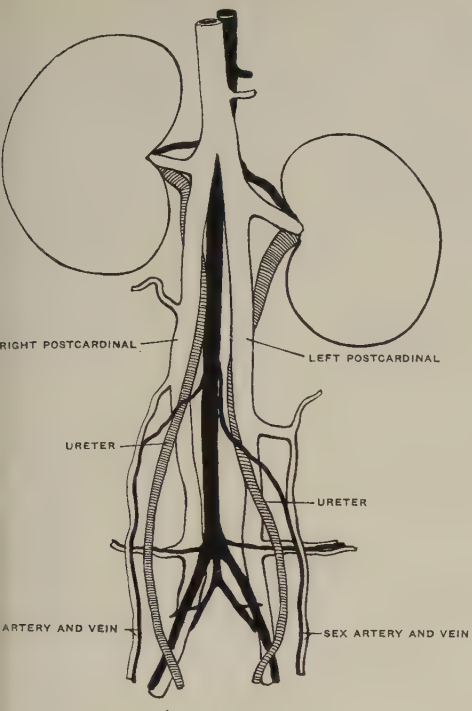
PLATE XV

EXPLANATION OF FIGURES

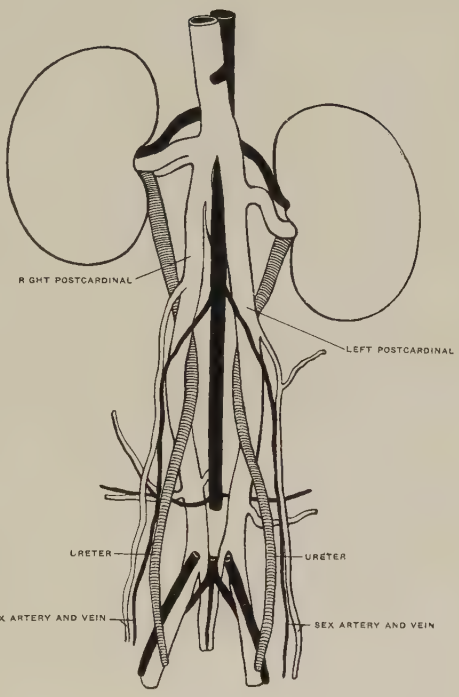
20 Adult cat (Darrach Series), *Type* AD. Persistence of right and left posterior cardinal veins. Ureters pass between aorta and caval veins.

21 Adult cat (Darrach Series), *Type* AD. Persistence of right and left posterior cardinal veins. Ureters pass between aorta and caval veins.

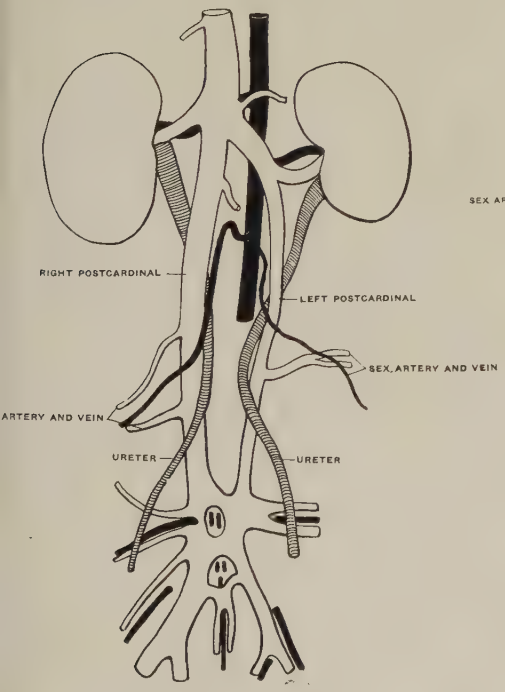
22 Adult cat (Darrach Series), *Type* AD. Persistence of right and left posterior cardinal veins. Ureters pass between aorta and caval veins.


RIGHT POSTCARDINAL
LEFT POSTCARDINAL
URETER
URETER
ARTERY AND VEIN
SEX ARTERY AND VEIN

20


R GHT POSTCARDINAL
LEFT POSTCARDINAL
URETER
URETER
SEX ARTERY AND VEIN
SEX ARTERY AND VEIN

21


RIGHT POSTCARDINAL
LEFT POSTCARDINAL
SEX. ARTERY AND VEIN
ARTERY AND VEIN
URETER
URETER

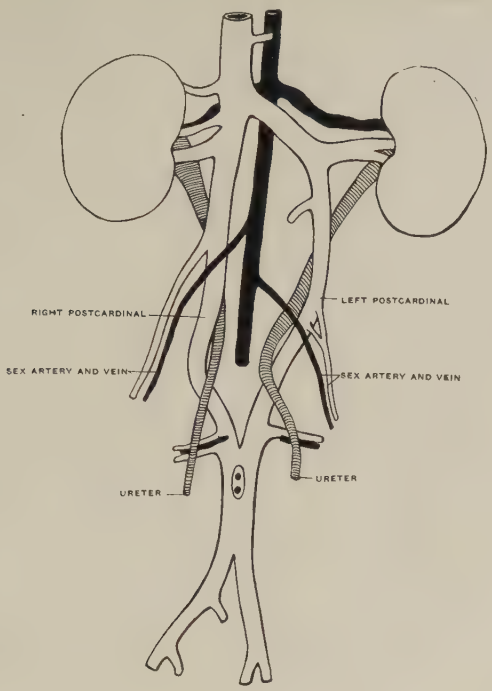
22

PLATE XVI

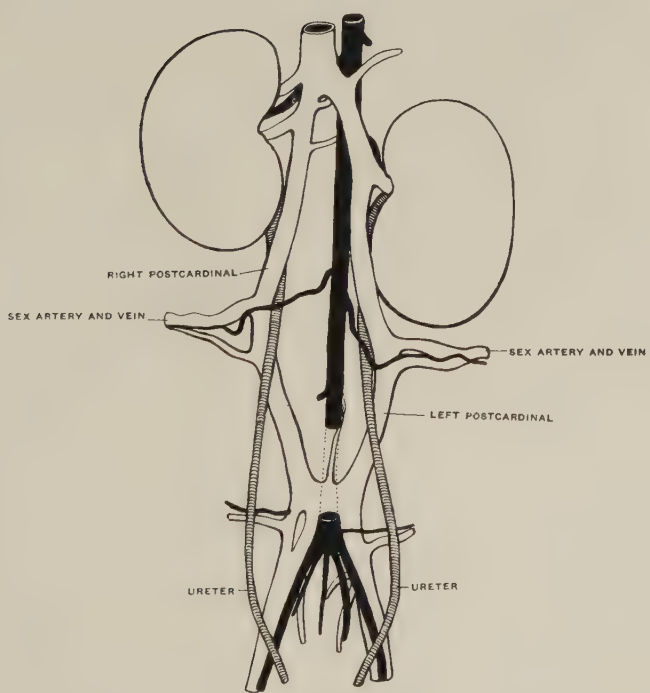
EXPLANATION OF FIGURES

23 Adult cat (Darrach Series), *Type* AD. Persistence of right and left posterior cardinal veins. Ureters pass between aorta and caval veins.

24 Adult cat (Darrach Series), *Type* AD. Persistence of right and left posterior cardinal veins. Ureters pass between aorta and caval veins.


RIGHT POSTCARDINAL
LEFT POSTCARDINAL
SEX ARTERY AND VEIN
SEX ARTERY AND VEIN
URETER
URETER

23

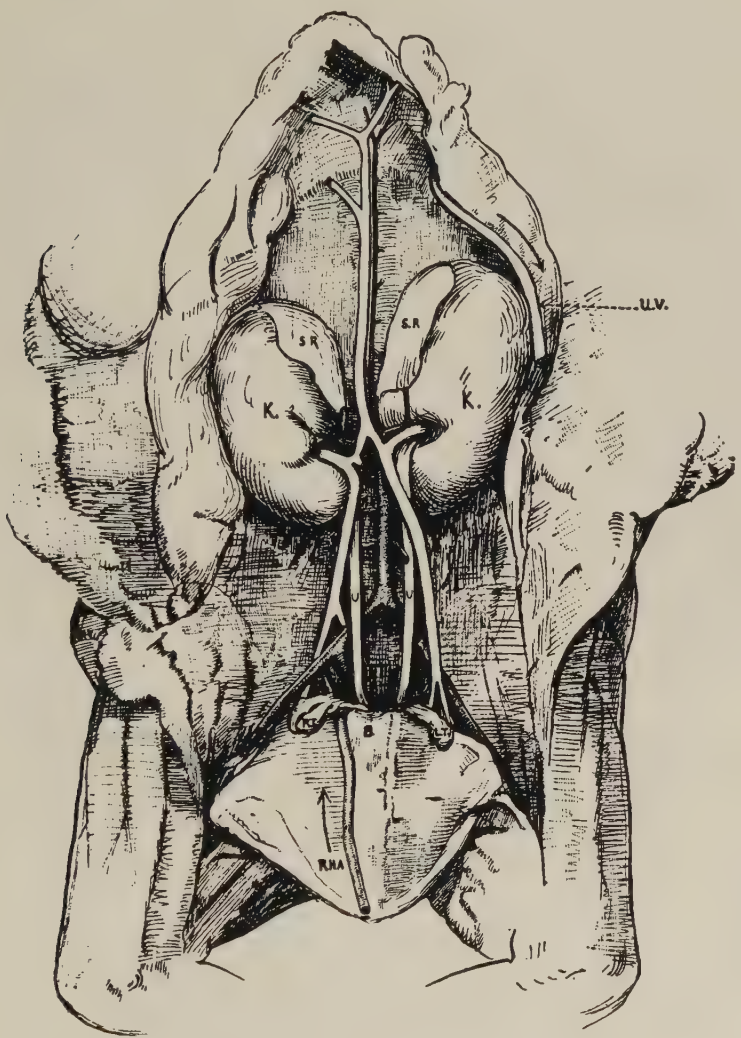

RIGHT POSTCARDINAL
SEX ARTERY AND VEIN
SEX ARTERY AND VEIN
LEFT POSTCARDINAL
URETER
URETER

24

PLATE XVII

EXPLANATION OF FIGURE

25 Man, acardiac foetus (from Gladstone, fig. 8, '12), *Type* AD. Persistence of right and left posterior cardinal veins. Ureters (*U*) pass between aorta and caval veins. Legends by Gladstone.



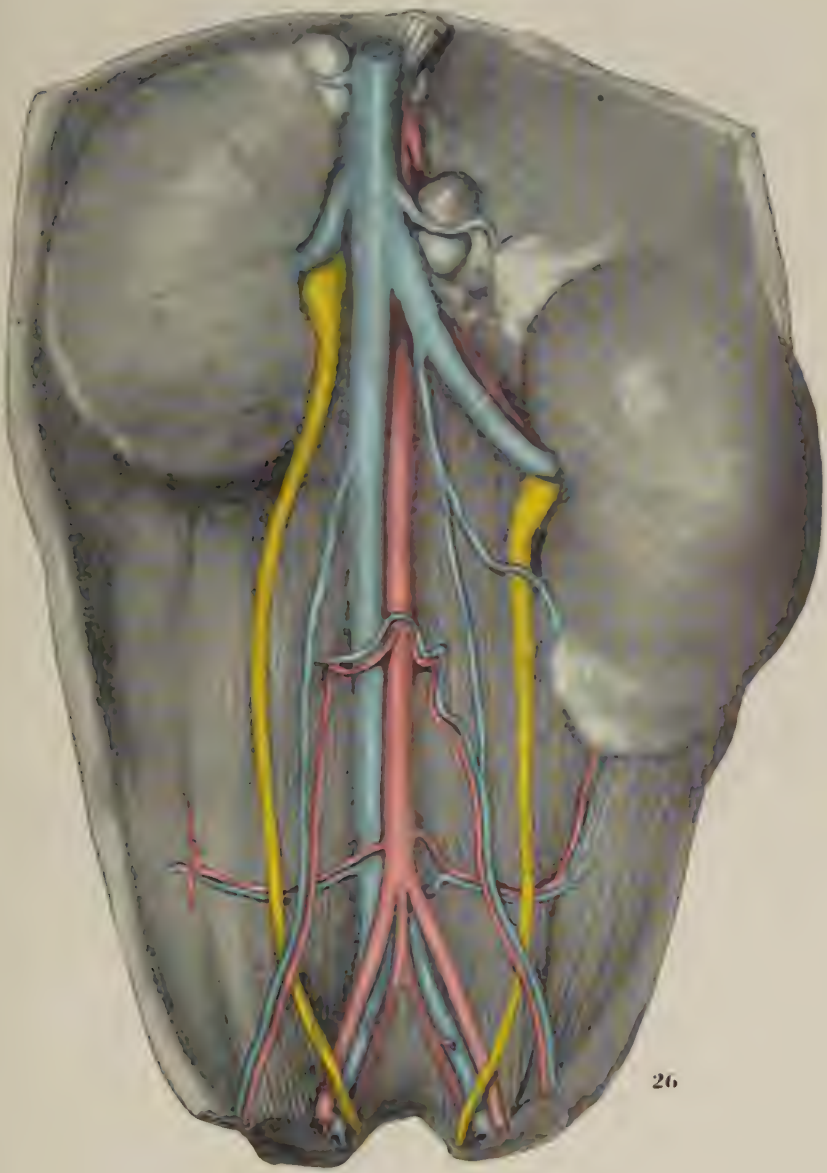
25

Drawing of the abdominal blood-vessels of an acardiac foetus, in which the ureter on each side passed behind the principal vein.

PLATE XVIII

EXPLANATION OF FIGURE

26 Adult cat (Darrach Series), *Type* B. Persistence of right supracardinal vein. Normal type of vena cava posterior (inferior) in the adult cat and in man. An atypical anastomosis present between Vv. spermaticae internae of opposite sides.



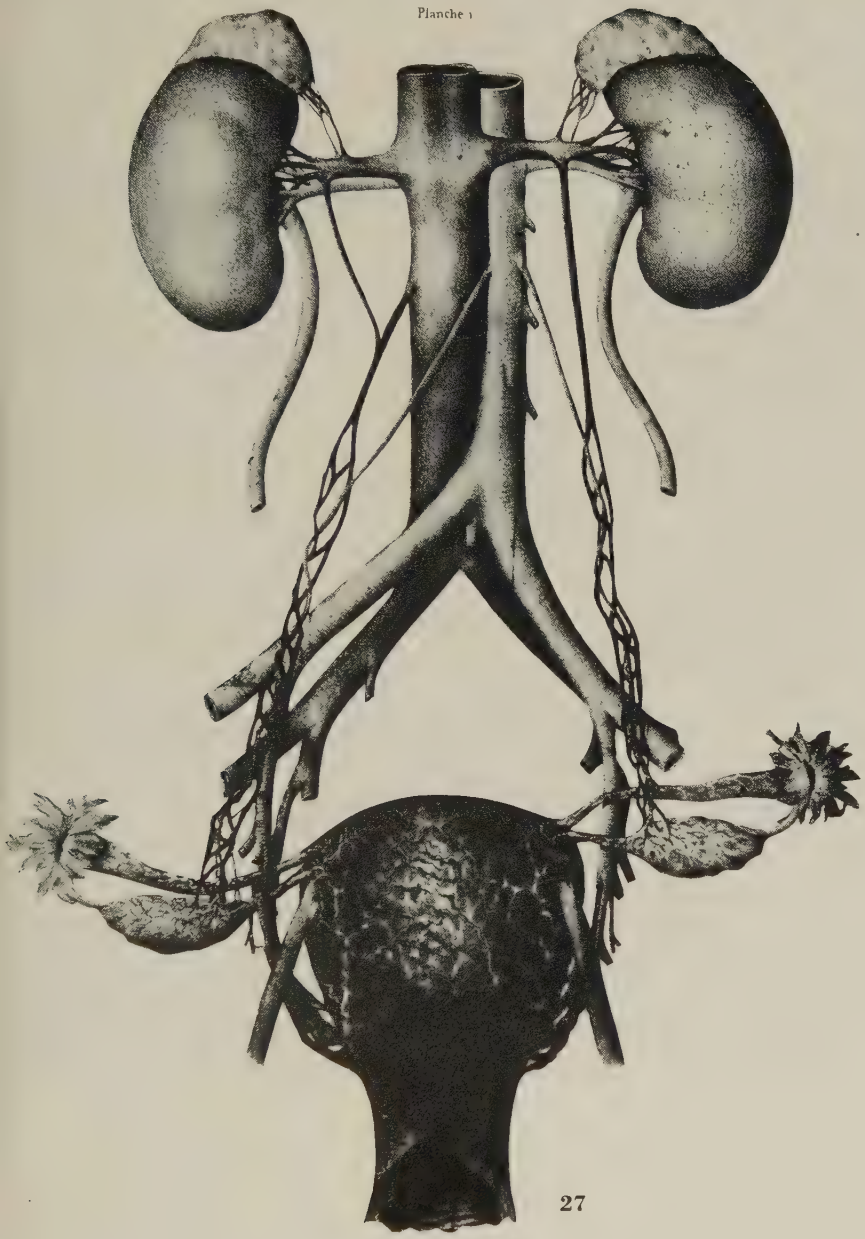
26

PLATE XIX

EXPLANATION OF FIGURE

27 Adult man (from Flourens, pl. 1, '36), *Type* B. Persistence of right supracardinal vein. Normal type of vena cava inferior in adult man. Atypical connection of V. spermatica dextra with V. renalis dextra.

Planche 1

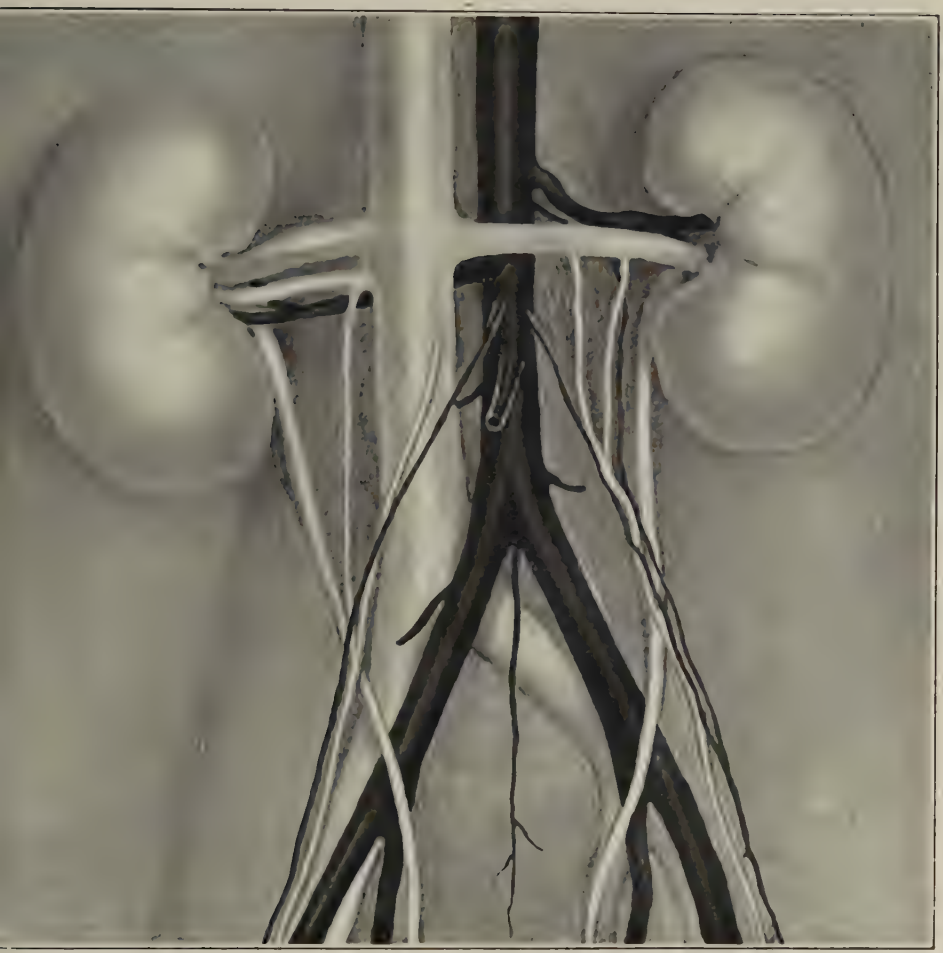


27

PLATE XX

EXPLANATION OF FIGURE

28 Adult man (Huntington Collection), *Type* B. Persistence of right supracardinal vein. Normal type of vena cava inferior in adult man. Atypical relations of vena spermatica on right and left sides.

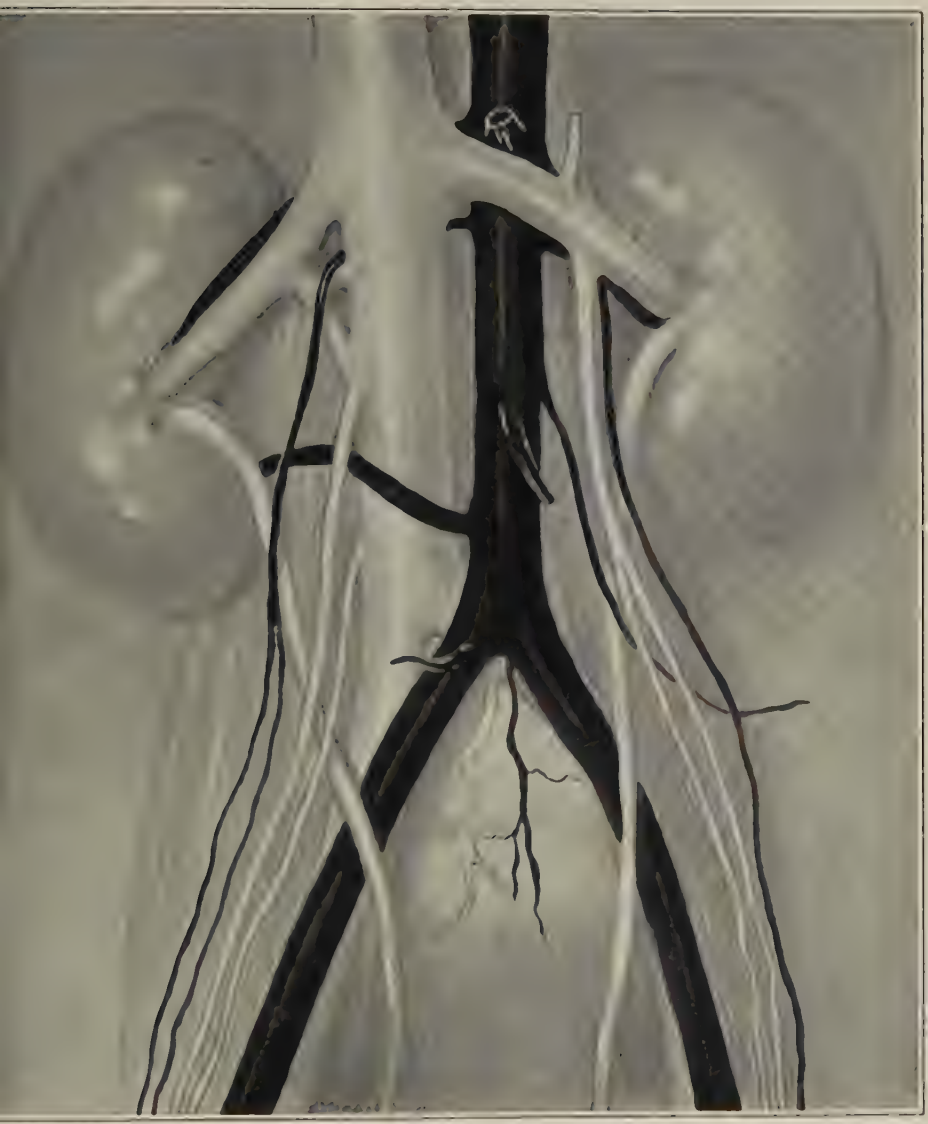


28

PLATE XXI

EXPLANATION OF FIGURE

29 Adult man (Huntington Collection), *Type* B. Persistence of right supracardinal vein. Normal type of vena cava inferior in adult man. Atypical relations of vena spermatica on right side.



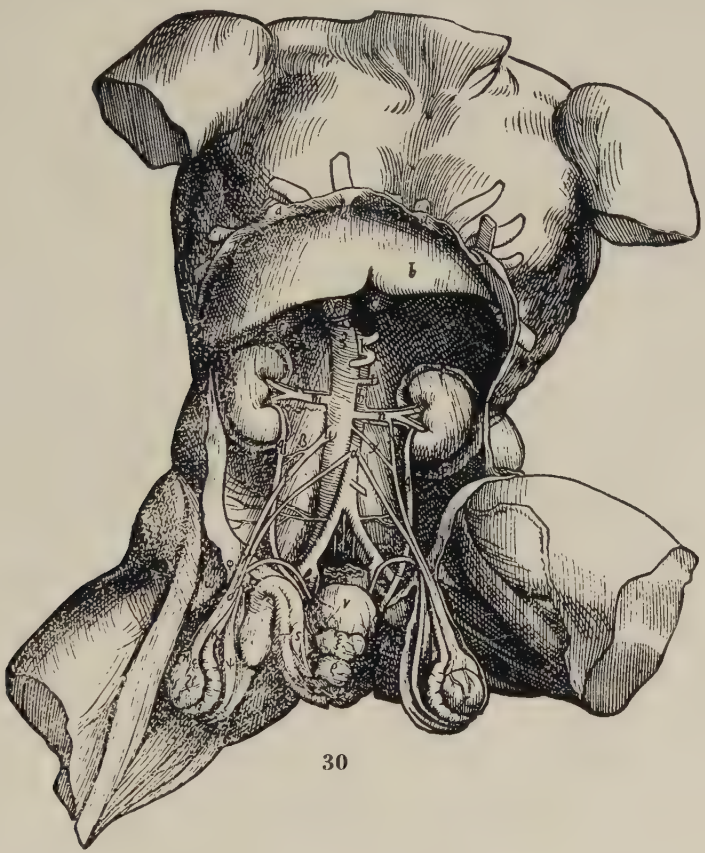
29

PLATE XXII

EXPLANATION OF FIGURE

30 Adult man (from Vesalius, 1543), *Type* B. Normal type of vena cava inferior in adult man. Atypical relations of vena spermatica sinistra.

IGESIMASECVNDA QVINTI LIBRI FIGVRA·



30

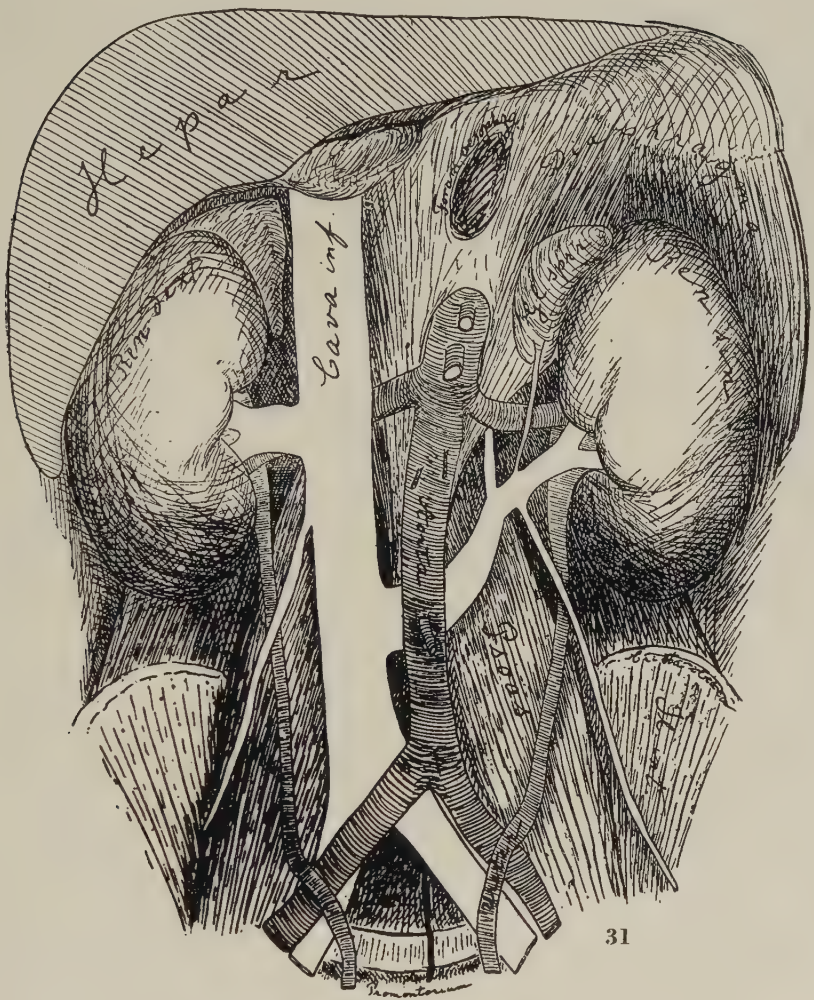
VIGE·

Venula à uenæ cauæ caudice pronata, ac uenæ ſeminali ſiniſtræ commixta unitá uc. Quamuis nim hæc uenula rariùs occurrat, tamen quum in alijs figuris ſiniſtra uena abſque hac delinea- ur, illaq; hic nihil obſcuret, ipſam exprimere uiſum eſt.

PLATE XXIII

EXPLANATION OF FIGURE

31 Adult man (from Froriep, '95), *Type* B. Persistence of right supra-cardinal vein. Normal type of vena cava inferior in adult man. Atypical vena renalis sinistra. Legends by Froriep.

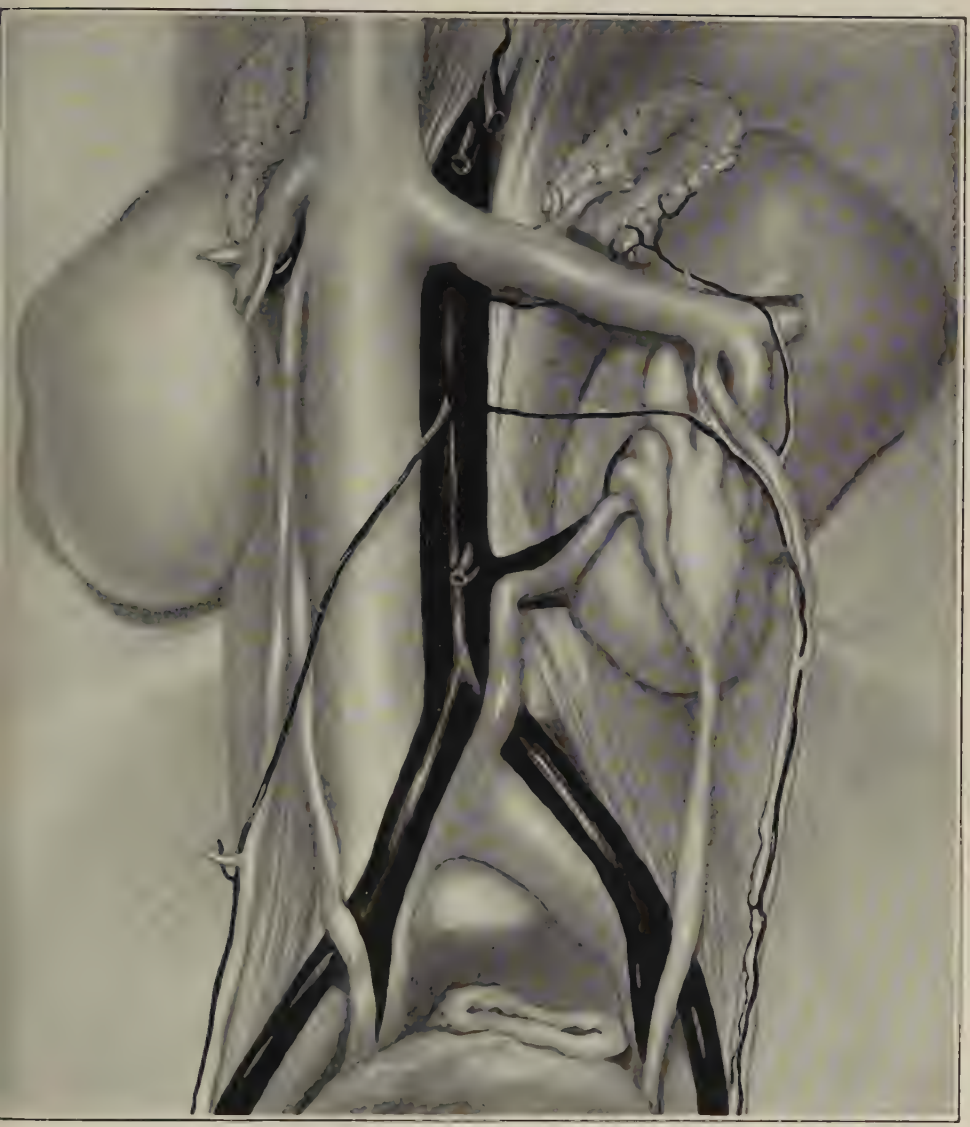


Fall I, nach dem Präparat gez. von AUGUST FROBIEP, halbe natürl. Größe. Die vordere Hälfte der Leber war durch einen frontalen Schnitt abgetragen und alle übrigen intraperitonealen Eingeweide vollständig entfernt. Die Venen sind in der Zeichnung unschraffirt gelassen, ihre Erklärung siehe im Text.

PLATE XXIV

EXPLANATION OF FIGURE

32 Adult man (Huntington Collection), *Type* B. Persistence of right supracardinal vein. Normal type of vena cava inferior in adult man. Atypical accessory vena renalis sinistra.

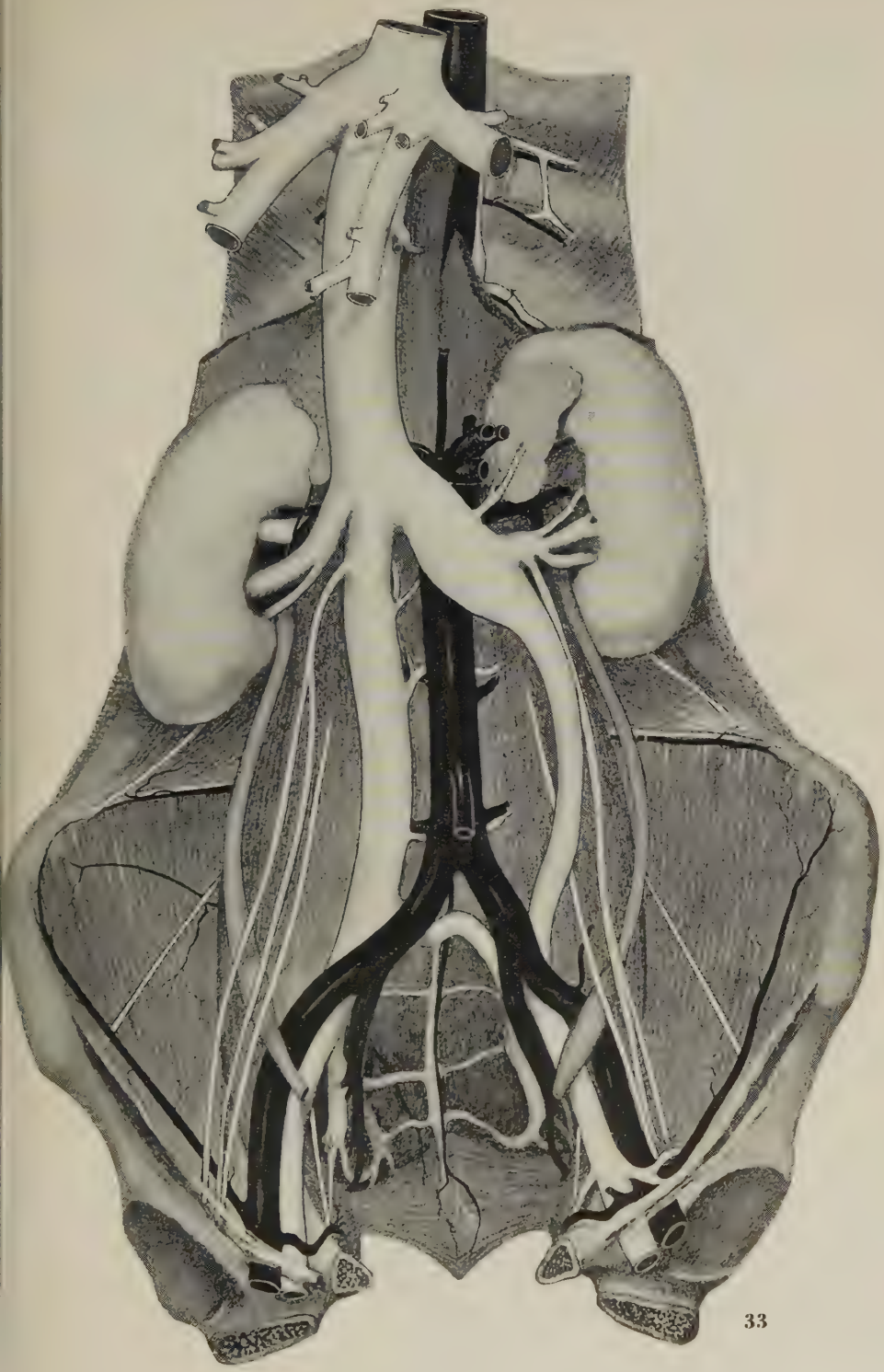


32

PLATE XXV

EXPLANATION OF FIGURE

33 Adult man (Huntington Collection), *Type* BC. Persistence of right and left supracardinal veins.

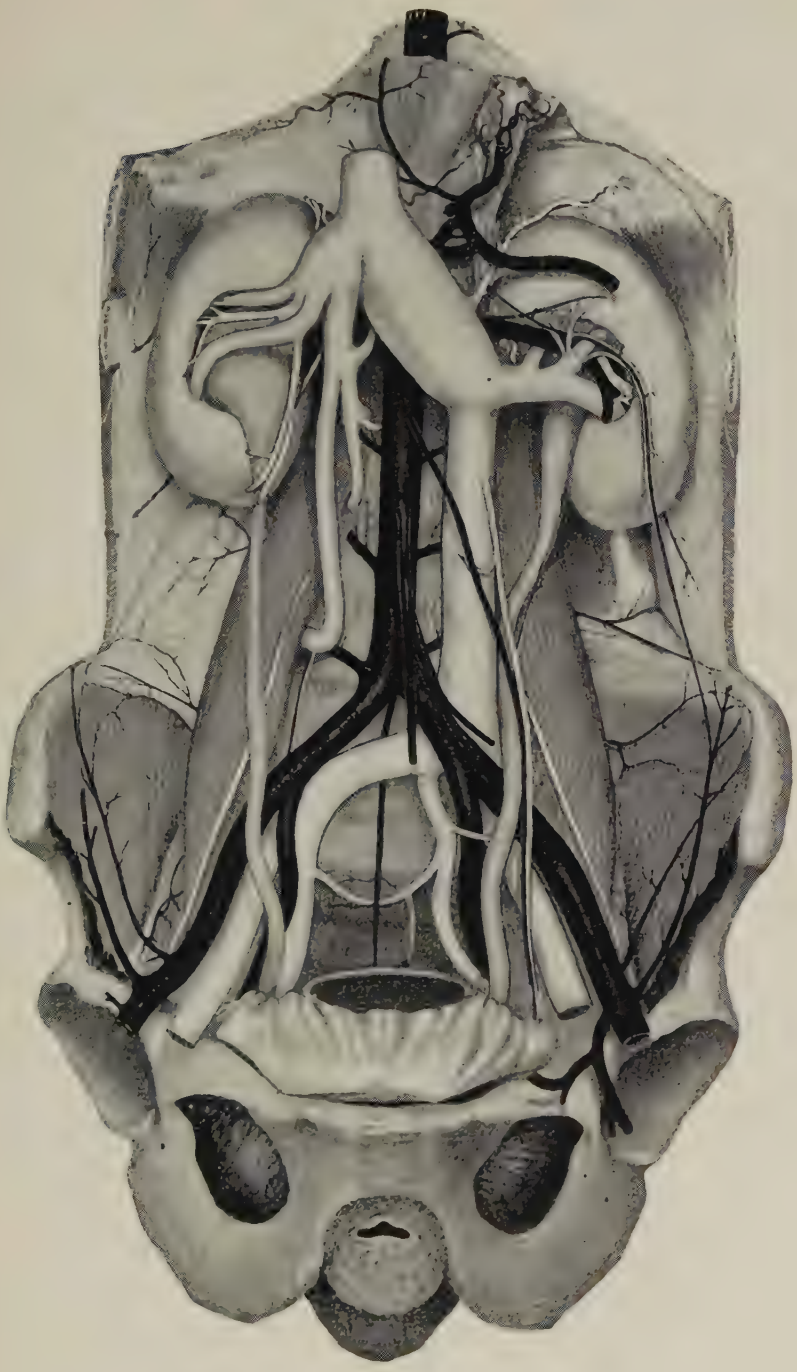


33

PLATE XXVI

EXPLANATION OF FIGURE

34 Adult man (Huntington Collection), *Type* BC. Persistence of right and left lumbar supracardinal veins. Caliber of caudal half of right cava much reduced in size.



34

PLATE XXVII

EXPLANATION OF FIGURE

35 Adult man (Huntington Collection), *Type* BC. Persistence of right and left lumbar supracardinal veins. Persistence of intersupracardinal anastomosis, dorsal to aorta, between supracardinal veins of opposite sides.

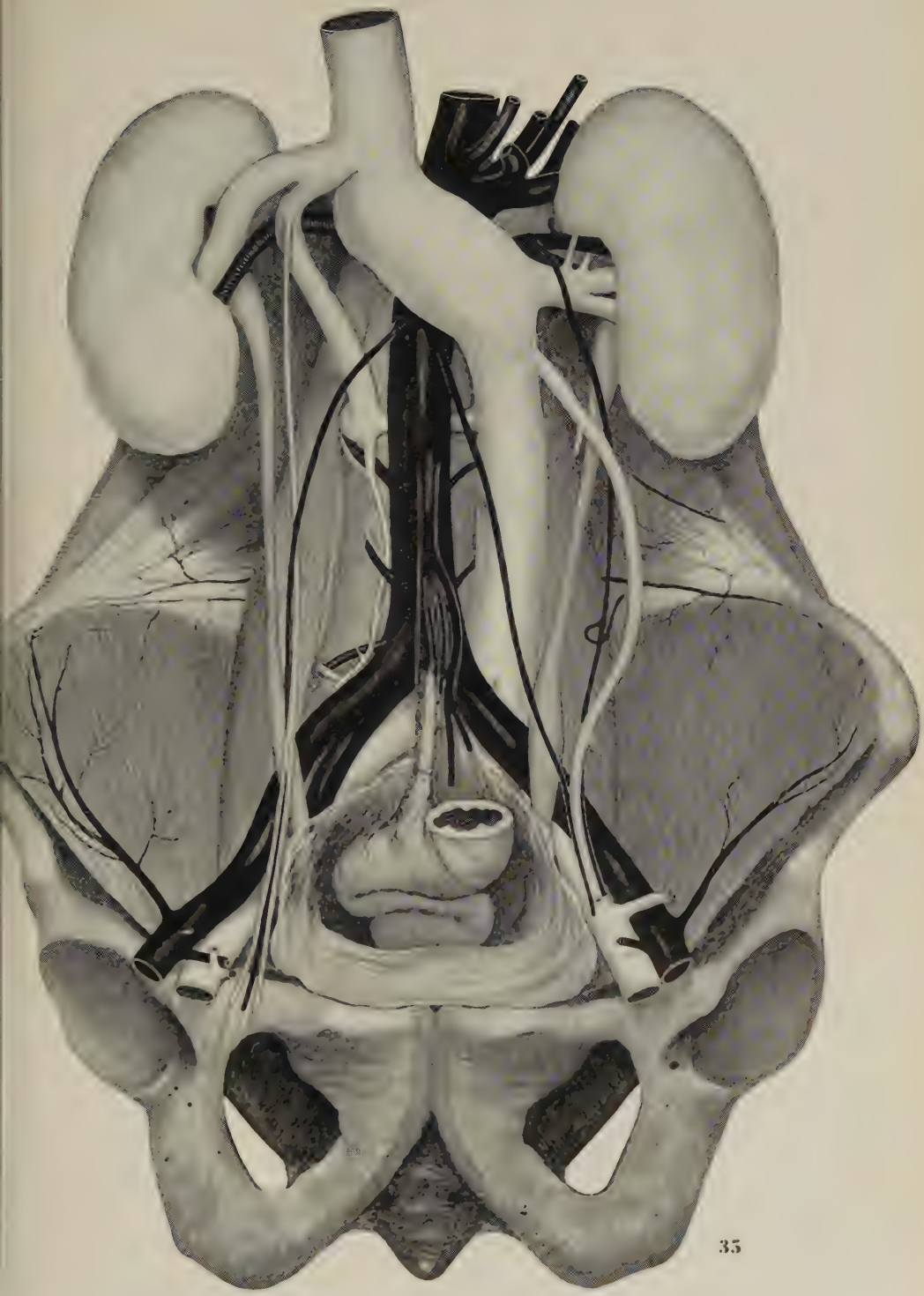
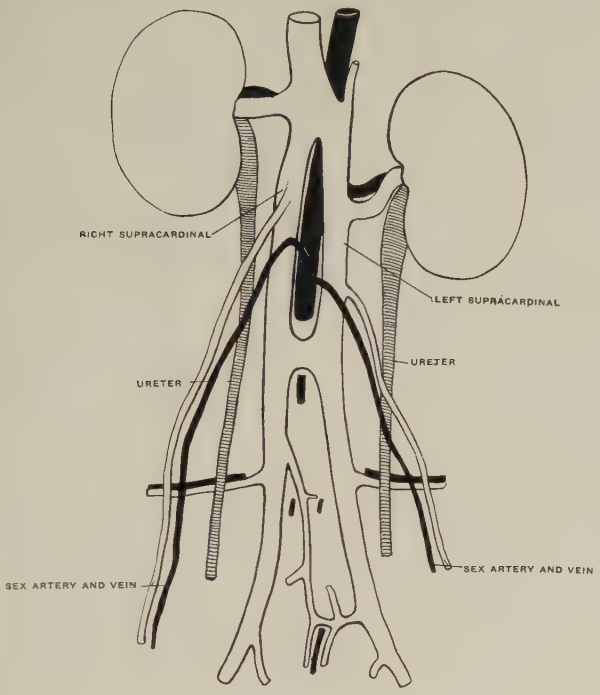

35

PLATE XXVIII

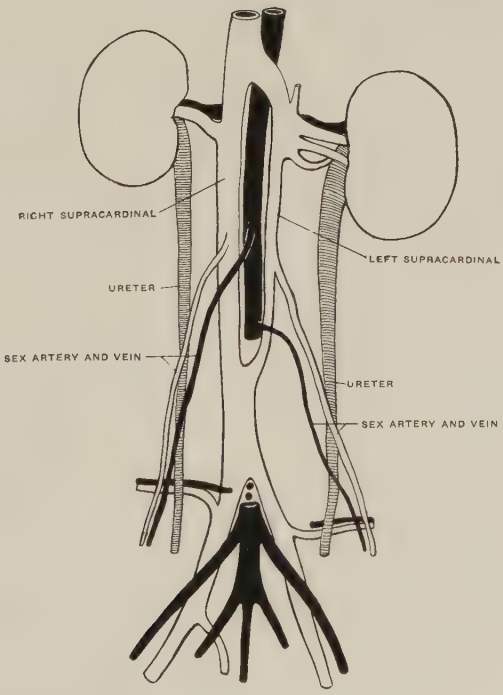
EXPLANATION OF FIGURES

36 Adult cat (Darrach Series), *Type* BC. Persistence of right and left supracardinal veins. Persistence of intersupracardinal anastomosis in midlumbar region, dorsal to aorta, between supracardinal veins of opposite sides.

37 Adult cat (Darrach Series), *Type* BC. Persistence of right supracardinal and cranial half of left lumbar supracardinal vein.


RIGHT SUPRACARDINAL
LEFT SUPRACARDINAL
URETER
URETER
SEX ARTERY AND VEIN
SEX ARTERY AND VEIN

36


RIGHT SUPRACARDINAL
LEFT SUPRACARDINAL
URETER
SEX ARTERY AND VEIN
URETER
SEX ARTERY AND VEIN

37

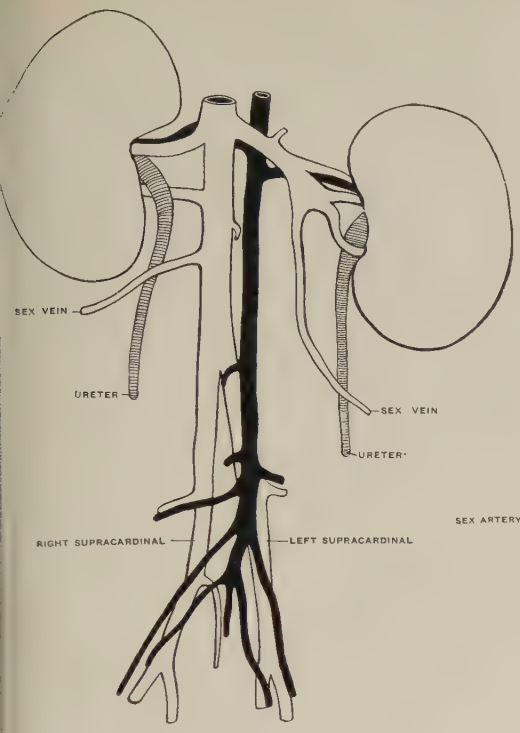
PLATE XXIX

EXPLANATION OF FIGURES

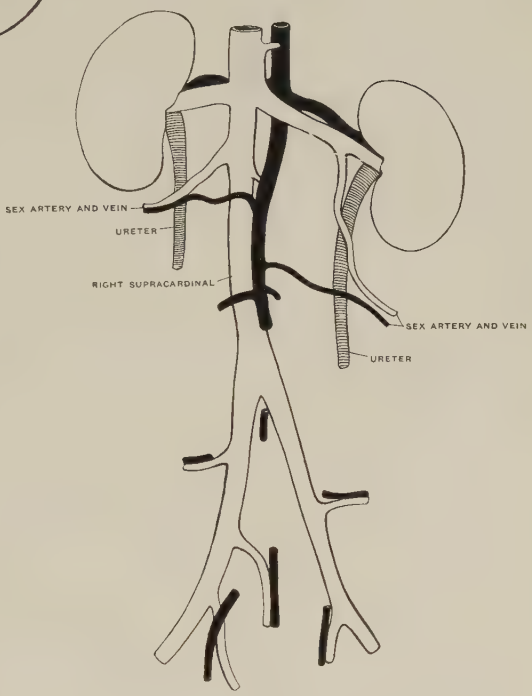
38 Adult cat (Darrach Series), *Type* BC. Persistence of right lumbar supracardinal and caudal section of left lumbar supracardinal vein.

39 Adult cat (Darrach Series), *Type* BC. Persistence of right lumbar supracardinal and caudal section of left lumbar supracardinal vein.

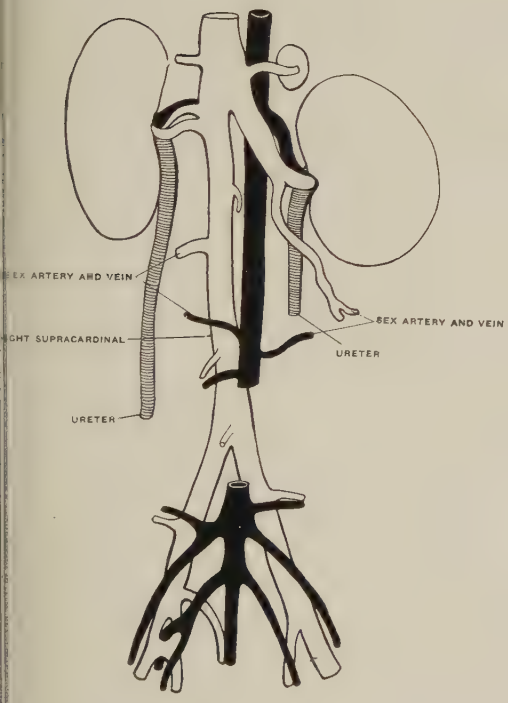
40 Adult cat (Darrach Series), *Type* BC. Persistence of right lumbar supracardinal and caudal section of left lumbar supracardinal vein.


SEX VEIN
URETER
SEX VEIN
URETER
RIGHT SUPRACARDINAL
LEFT SUPRACARDINAL

38


SEX ARTERY AND VEIN
URETER
RIGHT SUPRACARDINAL
SEX ARTERY AND VEIN
URETER

39


SEX ARTERY AND VEIN
SEX ARTERY AND VEIN
URETER
URETER

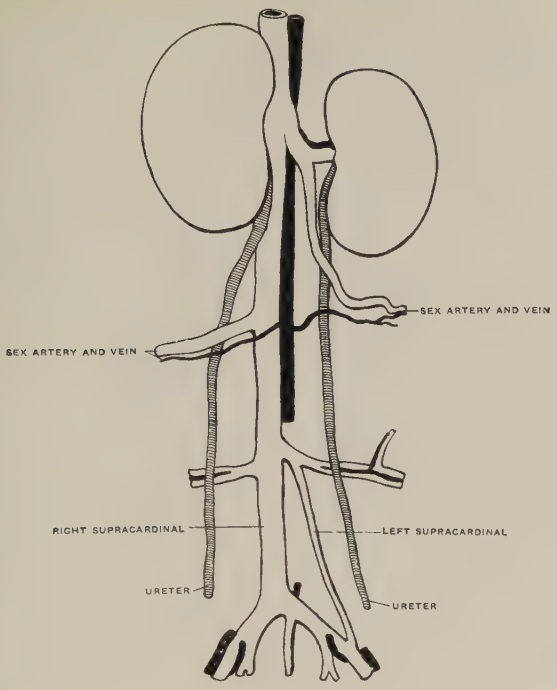
40

PLATE XXX

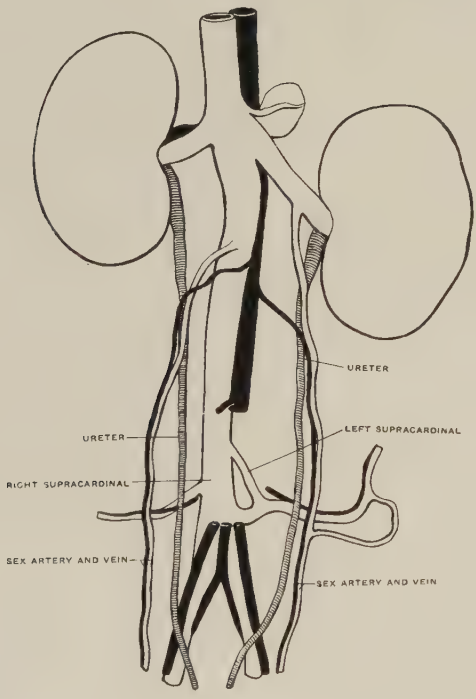
EXPLANATION OF FIGURES

41 Adult cat (Darrach Series), *Type* BC. Persistence of right lumbar supracardinal and caudal section of left lumbar supracardinal vein. Anastomosis present, dorsal to aorta, at iliac level, between iliac veins of opposite sides (left common iliac vein).

42 Adult cat (Darrach Series), *Type* BC. Persistence of right lumbar supracardinal and caudal section of left lumbar supracardinal vein. Anastomosis present, dorsal to aorta, at level of iliolumbar veins.


SEX ARTERY AND VEIN
SEX ARTERY AND VEIN
RIGHT SUPRACARDINAL
LEFT SUPRACARDINAL
URETER
URETER

41

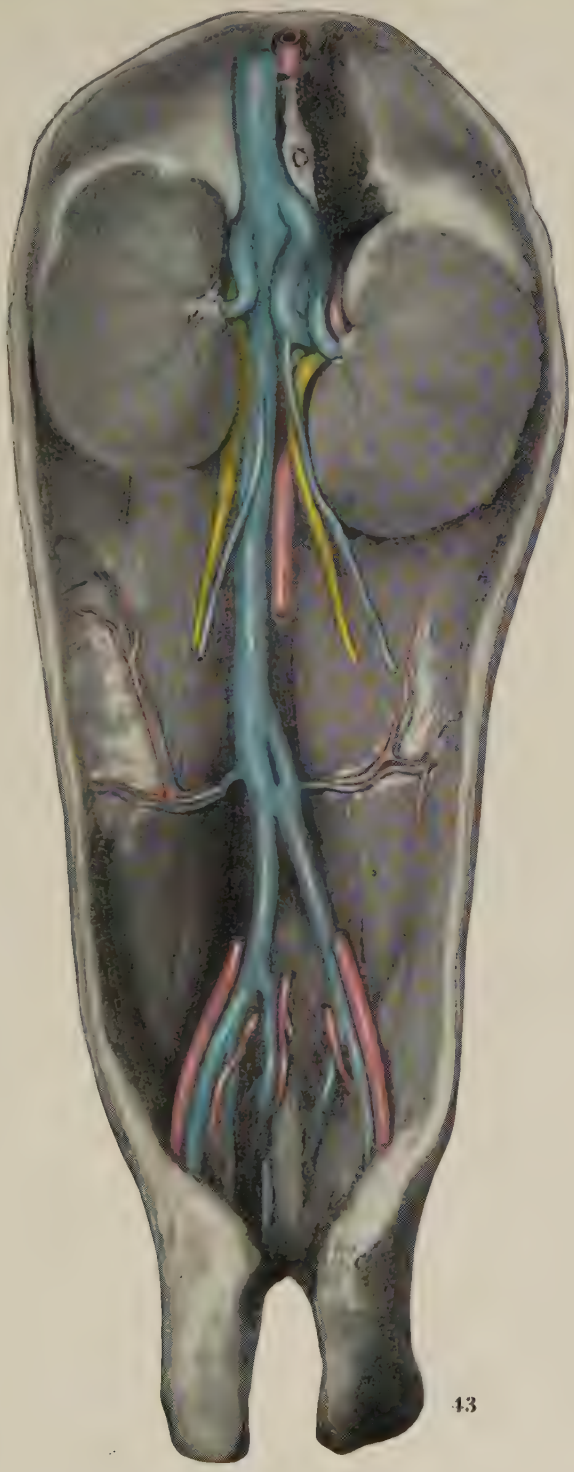

URETER
URETER
LEFT SUPRACARDINAL
RIGHT SUPRACARDINAL
SEX ARTERY AND VEIN
SEX ARTERY AND VEIN

42

PLATE XXXI

EXPLANATION OF FIGURE

43 Adult cat (Darrach Series), *Type* BC. Persistence of right lumbar supracardinal and caudal section of left lumbar supracardinal vein. Anastomosis present, dorsal to aorta, at level of iliolumbar veins.



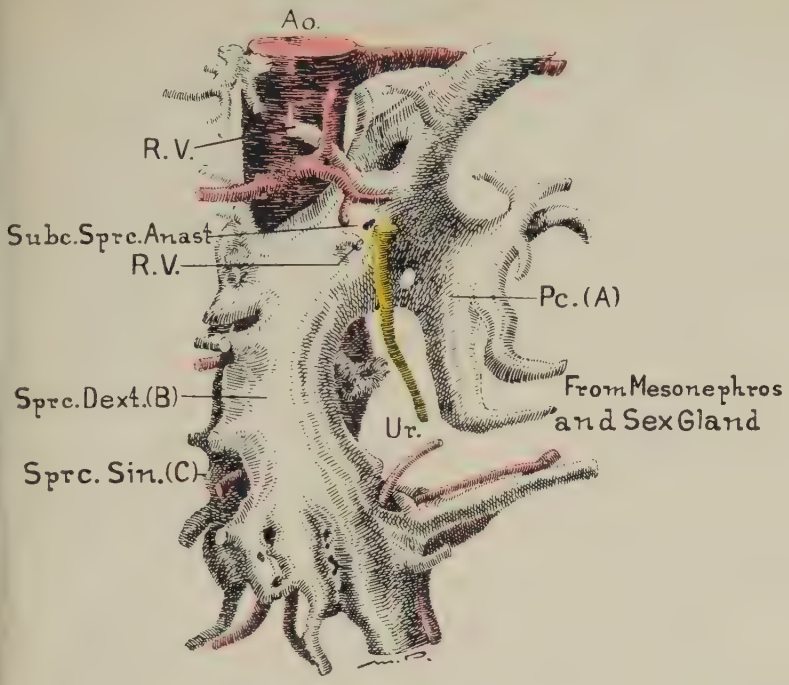
43

PLATE XXXII

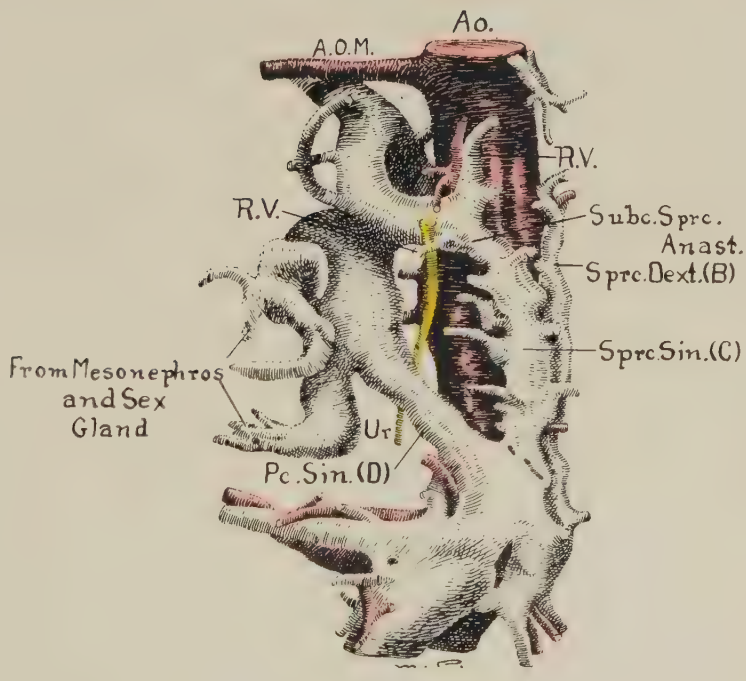
EXPLANATION OF FIGURES

44 Reconstruction (after Born) of veins in the lumbar region of a 17-mm. cat embryo. Lateral aspect, *right* side. Princeton Embryological Collection, no. 36.

45 Reconstruction (after Born) of veins in the lumbar region of a 17-mm. cat embryo. Lateral aspect, *left* side. Periureteric venous ring, through which ureter passes, present on the left side. In its present transition stage, the vena cava posterior is represented by *Type* BCD (right and left supracardinal, and left posterior cardinal veins). Princeton Embryological Collection, no. 36.


Ao.
R.V.
Subc.Sprc.Anast.
R.V.
Pc.(A)
From Mesonephros
and Sex Gland
Sprc.Dext.(B)
Ur.
Sprc. Sin.(C)

44


Ao.
A.O.M.
R.V.
R.V.
Subc.Sprc.
Anast.
Sprc.Dext.(B)
Sprc Sin.(C)
From Mesonephros
and Sex
Gland
Ur
Pc.Sin.(D)

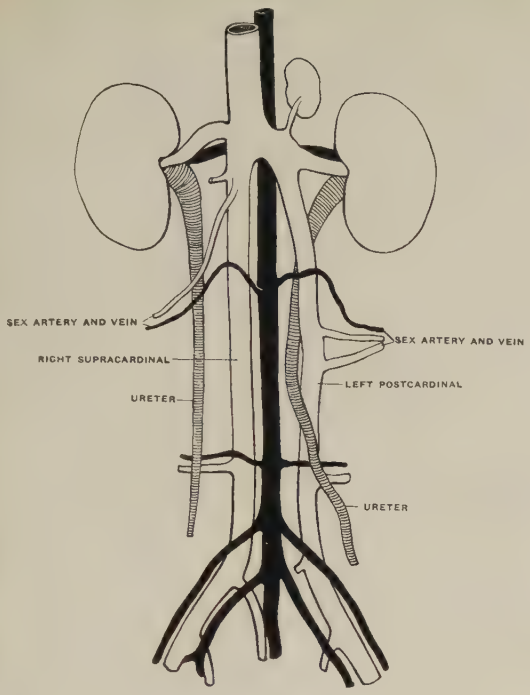
45

PLATE XXXIII

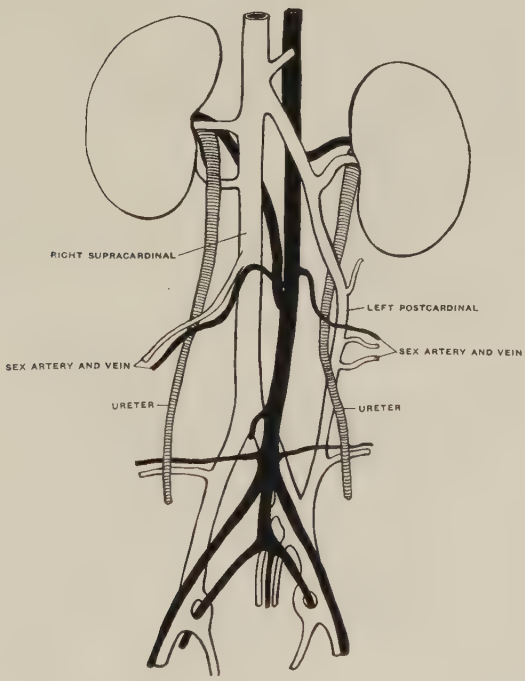
EXPLANATION OF FIGURES

46 Adult cat (Darrach Series), *Type* BD. Persistence of right supracardinal and left posterior cardinal veins.

47 Adult cat (Darrach Series), *Type* BD. Persistence of right supracardinal and left posterior cardinal veins.


SEX ARTERY AND VEIN
RIGHT SUPRACARDINAL
URETER
SEX ARTERY AND VEIN
LEFT POSTCARDINAL
URETER

46


RIGHT SUPRACARDINAL
SEX ARTERY AND VEIN
URETER
LEFT POSTCARDINAL
SEX ARTERY AND VEIN
URETER

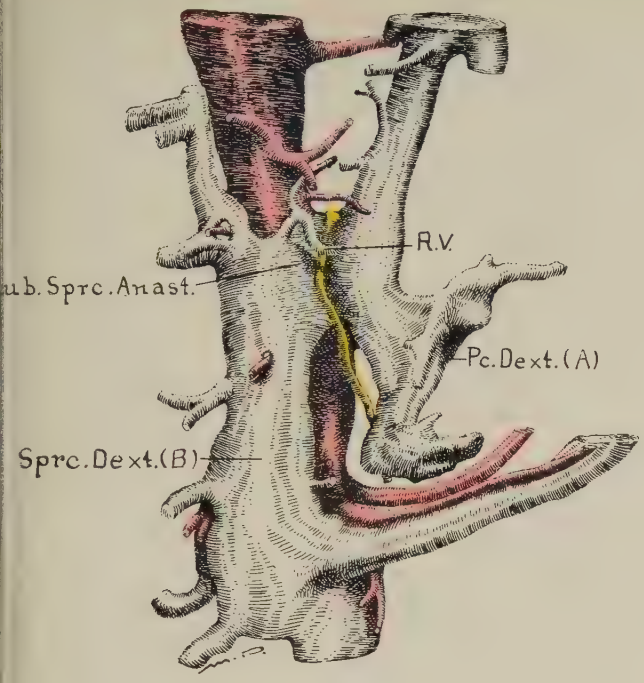
47

PLATE XXXIV

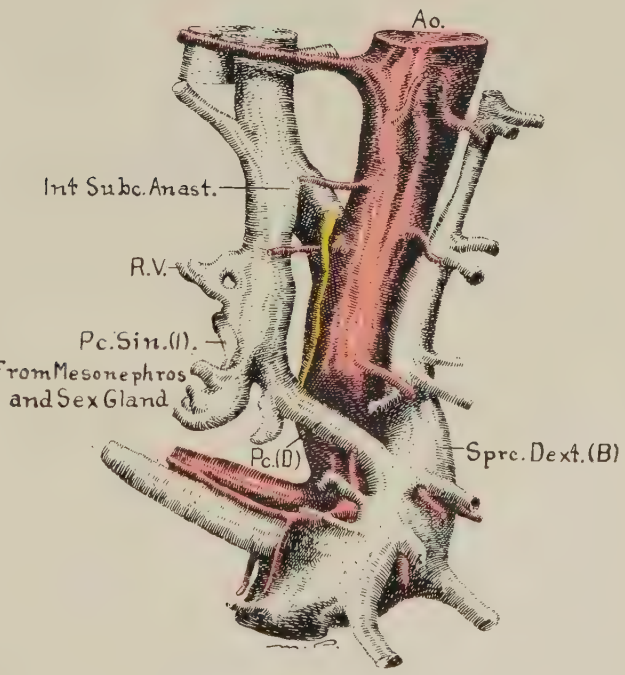
EXPLANATION OF FIGURES

48 Reconstruction (after Born) of veins in the lumbar region of a 23-mm. cat embryo. Lateral aspect, *right* side. Note presence of right side of renal collar (*Subc.Sprc.Anast.*) and fusion between right and left supracardinal veins. Coll. of P. and S. Embryological Collection, no. 569.

49 Reconstruction (after Born) of veins in the lumbar region of a 23-mm. cat embryo. Lateral aspect, *left* side. Note absence of left side of renal collar and presence of left posterior cardinal vein (*Pc.-D*). In its present transition stage, the vena cava is represented by *Type* BD (right supracardinal and left posterior cardinal veins). Coll. of P. and S. Embryological Collection, no. 569.


R.V.
ub. Sprc. Anast.
Pc. Dext. (A)
Sprc. Dext. (B)

48

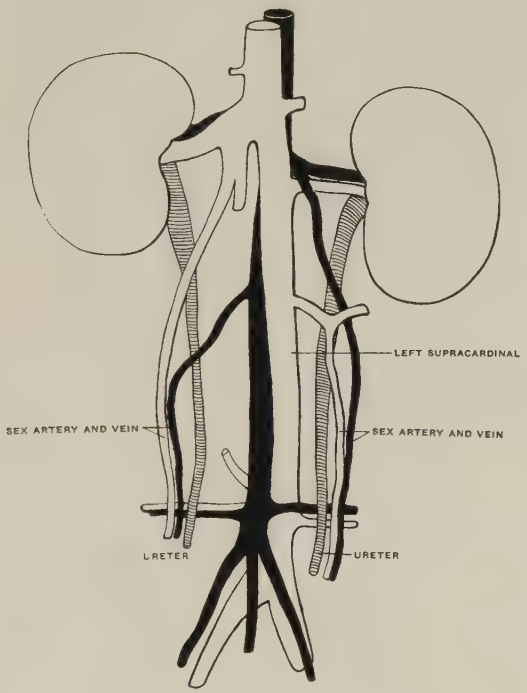

Ao.
Int Subc. Anast.
R.V.
Pc. Sin. (I).
From Mesonephros and Sex Gland
Pc. (D)
Sprc. Dext. (B)

49

PLATE XXXV

EXPLANATION OF FIGURE

50 Adult cat (Darrach Series), *Type* C. Persistence of left supracardinal vein.

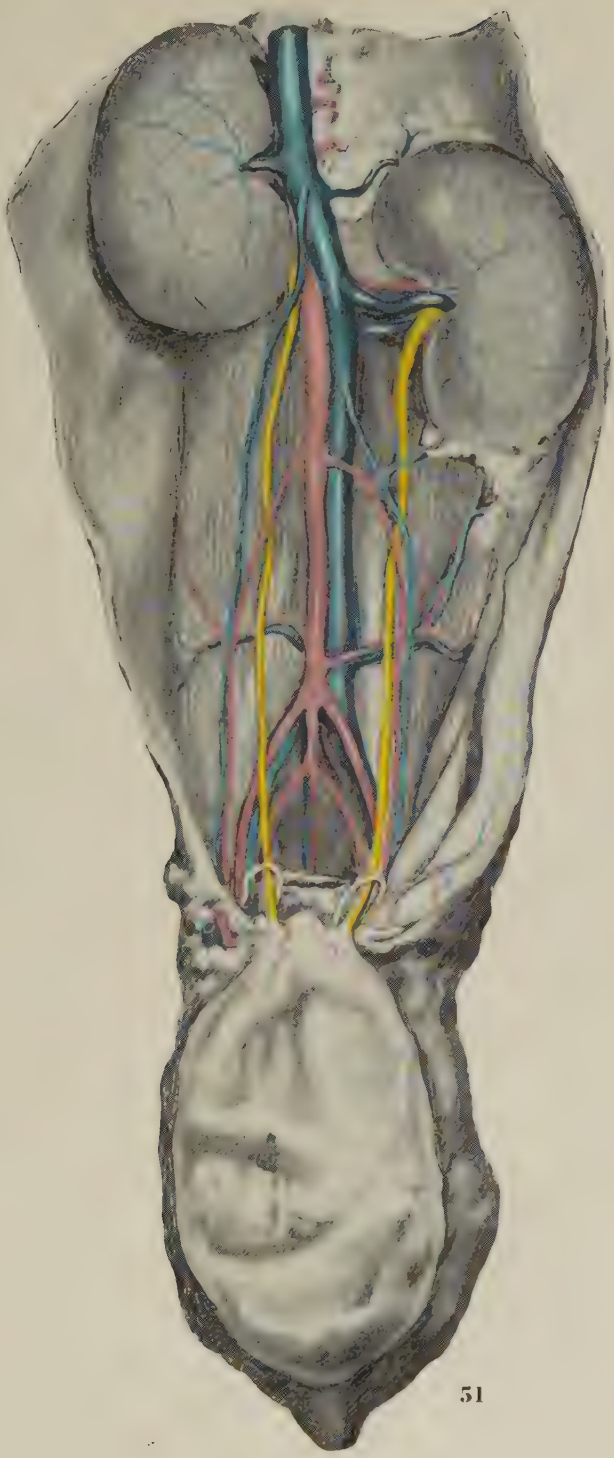

LEFT SUPRACARDINAL
SEX ARTERY AND VEIN
SEX ARTERY AND VEIN
URETER
URETER

50

PLATE XXXVI

EXPLANATION OF FIGURE

51 Adult cat (Darrach Series), *Type* C. Persistence of left supracardinal vein.



51

PLATE XXXVII

EXPLANATION OF FIGURE

52 Reconstruction (after Born) of veins in the lumbar region of a 45-mm. cat embryo. Lateral aspect, *left* side. *Type* C (left supracardinal vein). Note elongated left side of renal collar (*Subc.Sprc.Anast.*), which forms a segment of the vena cava; also, opening of V. spermatica interna (*Pc.*) into cava at caudal end of renal collar (*Subc.Sprc.Anast.*). Princeton Embryological Collection, no. 14.

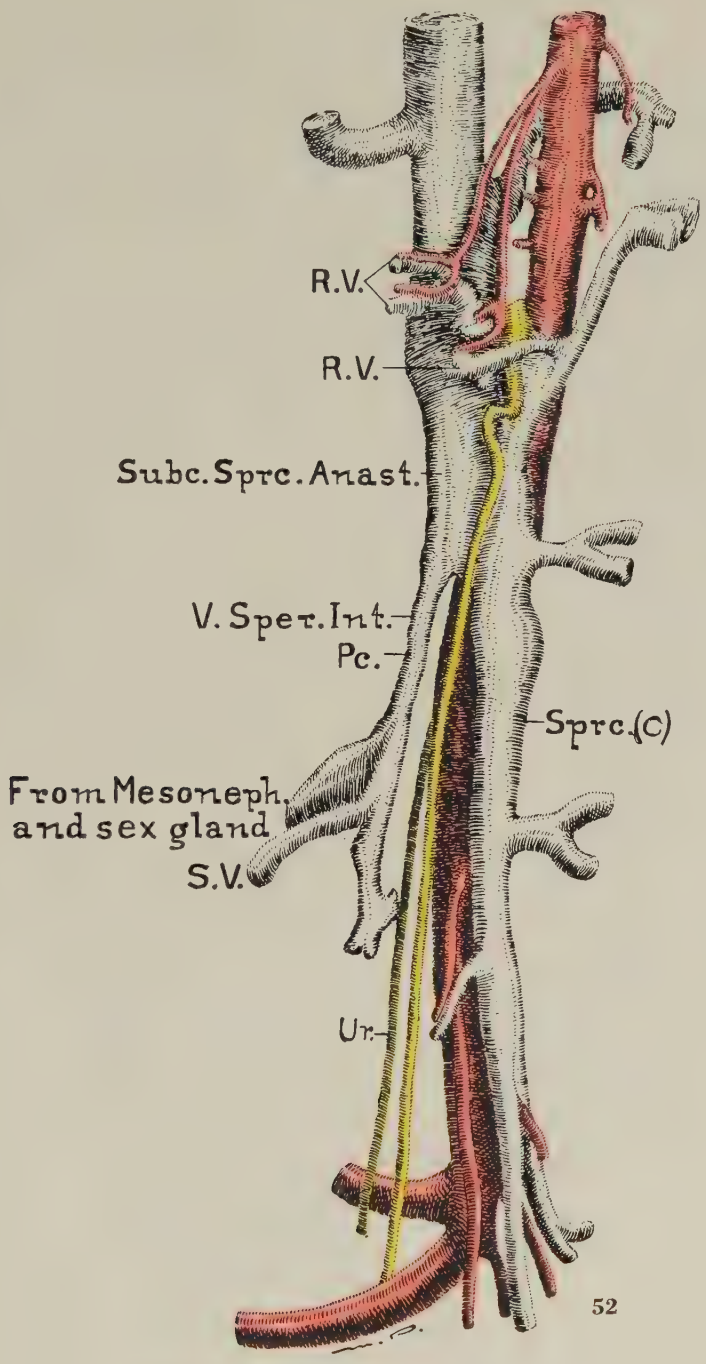

R.V.
R.V.
Subc. Sprc. Anast.
V. Sper. Int.
Pc.
Sprc. (C)
From Mesoneph. and sex gland
S.V.
Ur.
52

PLATE XXXVIII

EXPLANATION OF FIGURE

53 Adult cat (Darrach Series), *Type* D. Persistence of left posterior cardinal vein.

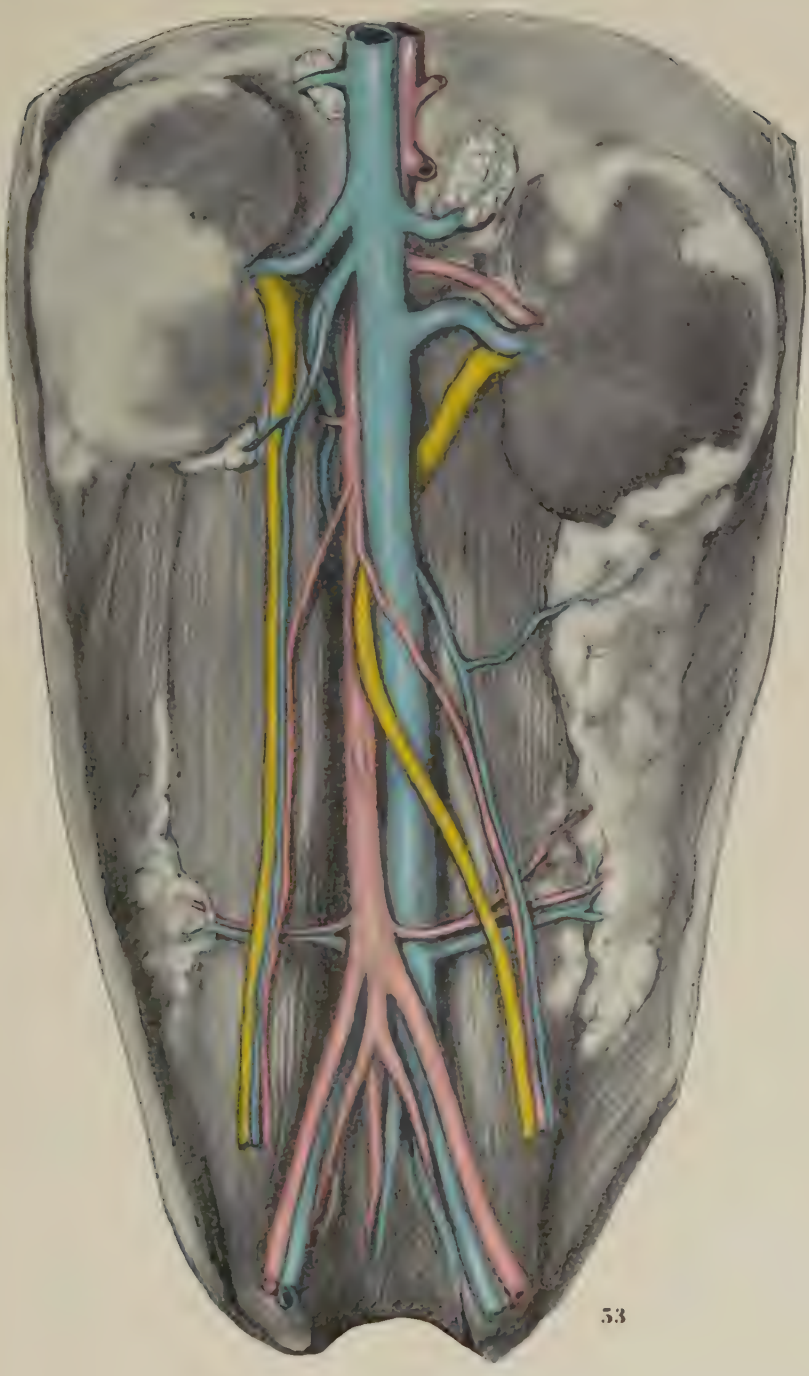
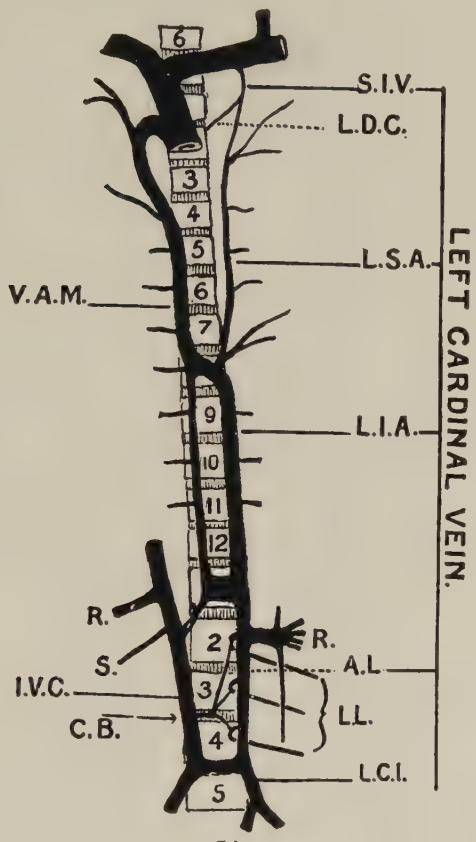

53

PLATE XXXIX

EXPLANATION OF FIGURE

54 Adult man (after Fawcett, fig. 5, '98). Persistence of embryonic supracardinal system of veins, continuous in lumbar and thoracic regions. Prerenal division of vena cava inferior also present. Legends by Fawcett.



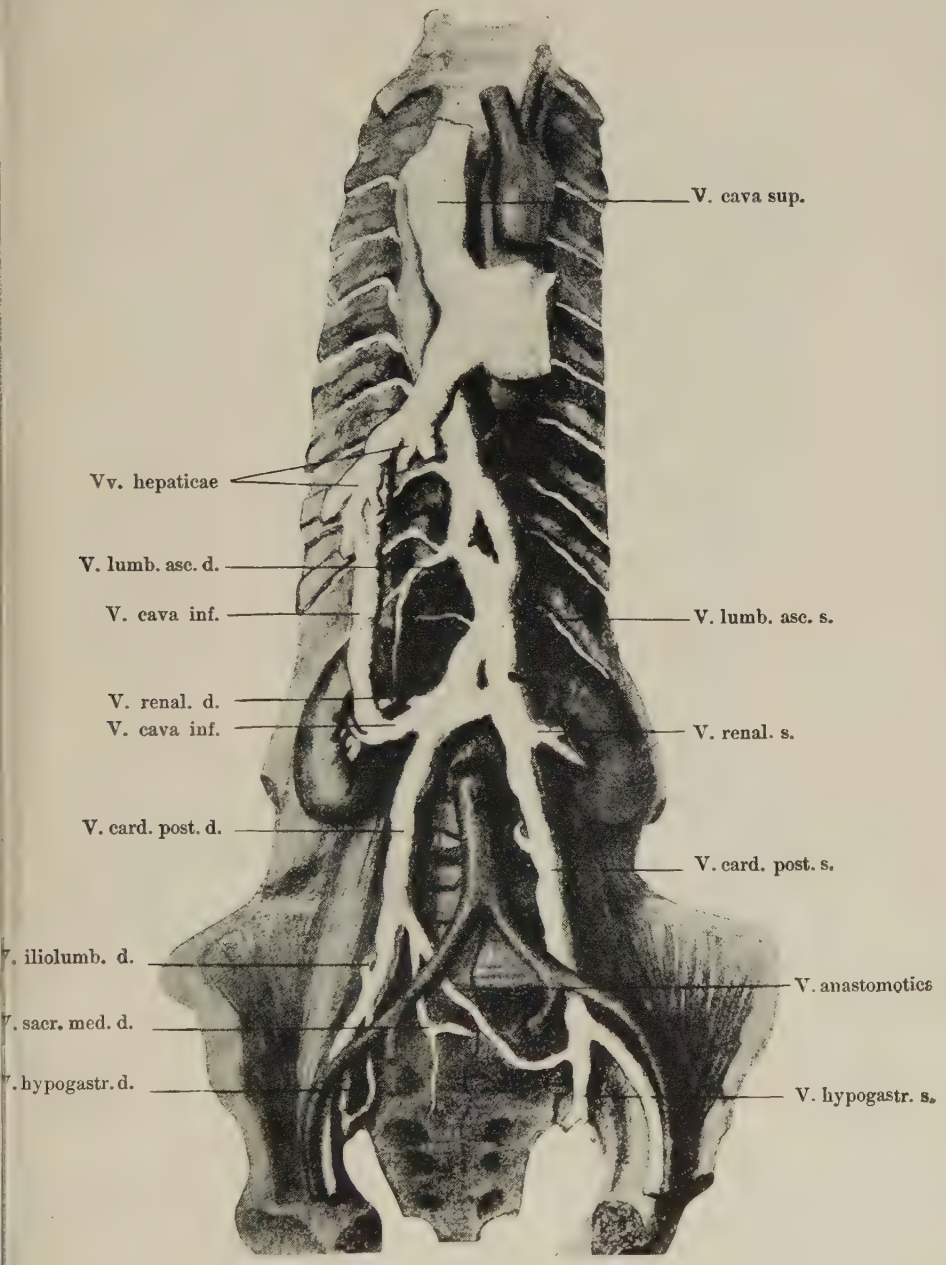
54

S.I.V., left sup. intercostal vein; *L.D.C.*, lower part of left duct of Cuvier; *L.S.A.*, left sup. azygos vein; *L.I.A.*, left inf. azygos vein; *R.R.*, right and left renal veins; *A.L.*, ascending lumbar; *L.L.*, left lumbar veins; *L.C.I.*, left common iliac; *C.B.*, common trunk uniting left lumbar veins and bringing them into communication with inf. vena cava; *I.V.C.*, inf. vena cava; *S.*, right spermatic artery; *V.A.M.*, vena azygos major.

PLATE XL

EXPLANATION OF FIGURE

55 Adult man (after Lauber, fig. 1, '01). Persistence of embryonic supracardinal system of veins, continuous in lumbar and thoracic regions. Prerenal division of vena cava inferior also present. Legends by Lauber.

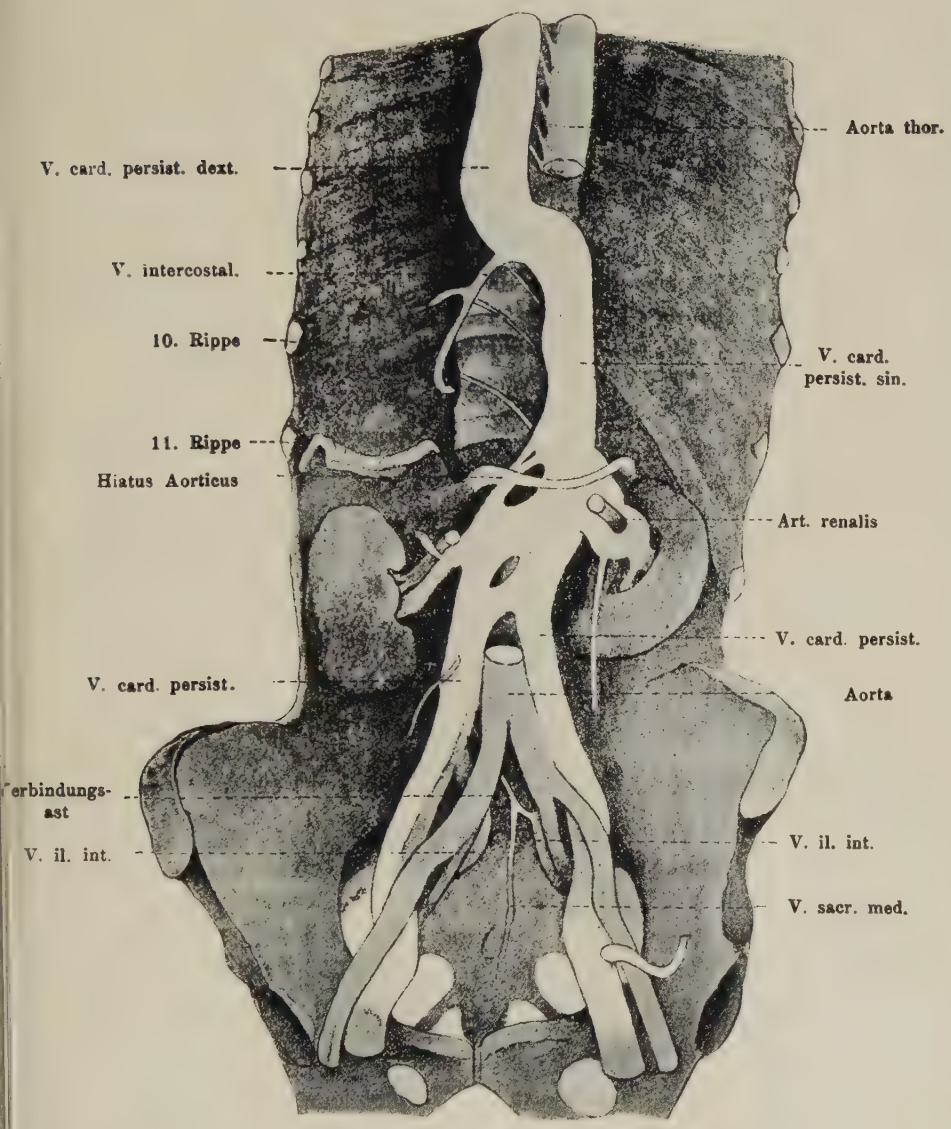

V. cava sup.
Vv. hepaticae
V. lumb. asc. d.
V. cava inf.
V. lumb. asc. s.
V. renal. d.
V. cava inf.
V. renal. s.
V. card. post. d.
V. card. post. s.
. iliolumb. d.
V. anastomotica
. sacr. med. d.
. hypogastr. d.
V. hypogastr. s.

55

PLATE XLI

EXPLANATION OF FIGURE

56 Adult man (after Kollmann, fig. 3, '93). Persistence of embryonic supra-cardinal system of veins, continuous in lumbar and thoracic regions. Prerenal division of vena cava inferior *absent*. Legends by Kollmann.

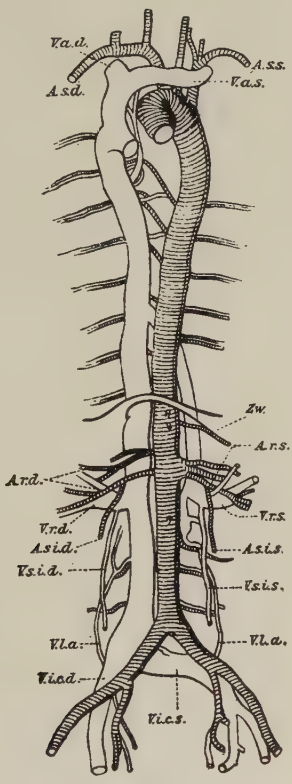

Aorta thor.
V. card. persist. dext.
V. intercostal.
10. Rippe
V. card. persist. sin.
11. Rippe
Hiatus Aorticus
Art. renalis
V. card. persist.
V. card. persist.
Aorta
Verbindungs-ast
V. il. int.
V. il. int.
V. sacr. med.

56

PLATE XLII

EXPLANATION OF FIGURE

57 Adult man (after Hochstetter, fig. 14, '93). Persistence of embryonic supracardinal system of veins (right side), continuous in lumbar and thoracic regions. Prerenal division of vena cava inferior *absent*. Legends by Hochstetter.

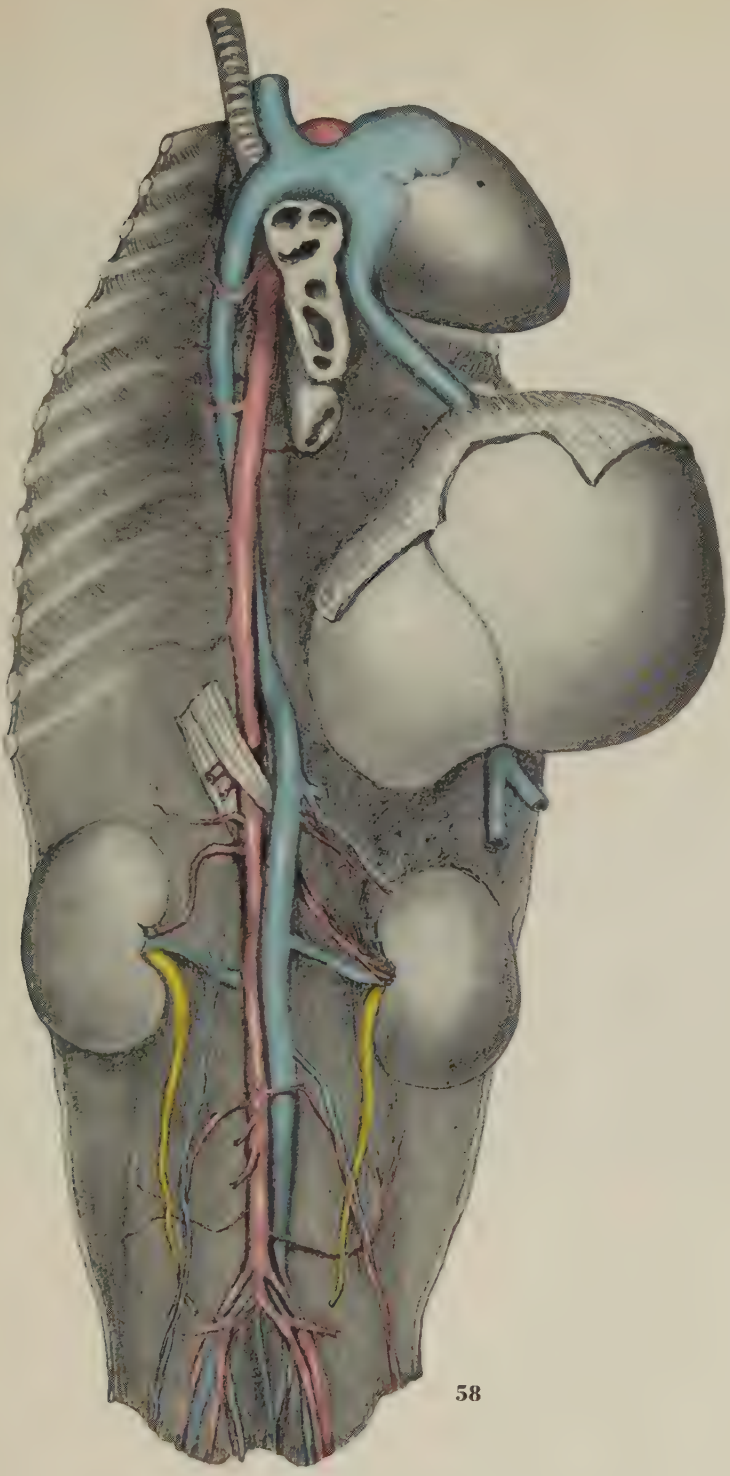

V.a.d.
A.s.d.
A.s.s.
V.a.s.
Zw.
A.r.s.
A.r.d.
V.r.s.
V.r.d.
A.s.i.d.
A.s.i.s.
V.s.i.d.
V.s.i.s.
V.l.a.
V.l.a.
V.i.c.d.
V.i.c.s.

57

PLATE XLIII

EXPLANATION OF FIGURE

58 Adult cat (Princeton Morphological Museum, no. 1040). Persistence of embryonic supracardinal system of veins, continuous in lumbar and thoracic regions. Prerenal division of vena cava posterior *absent*.

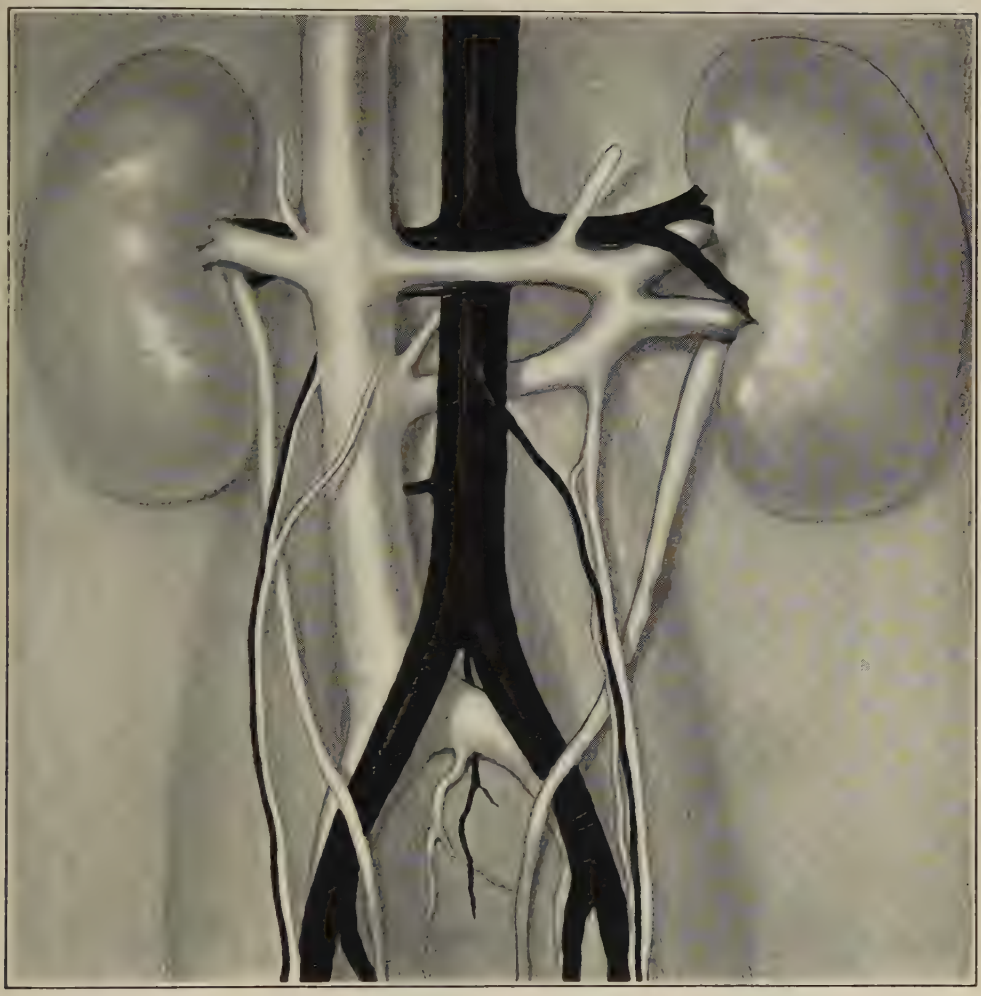


58

PLATE XLIV

EXPLANATION OF FIGURE

59 Adult man (Huntington Collection). Persistence of renal collar.



39

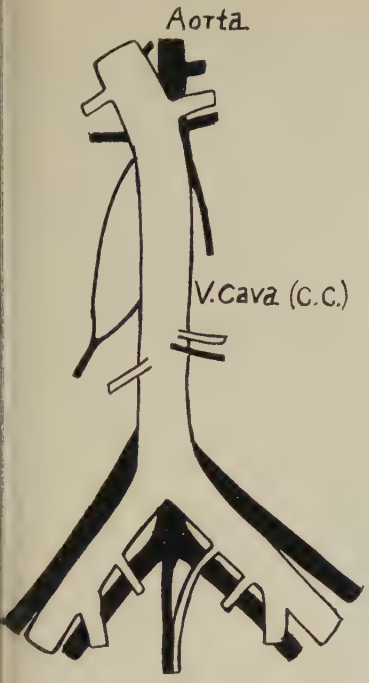
PLATE XLV

EXPLANATION OF FIGURES

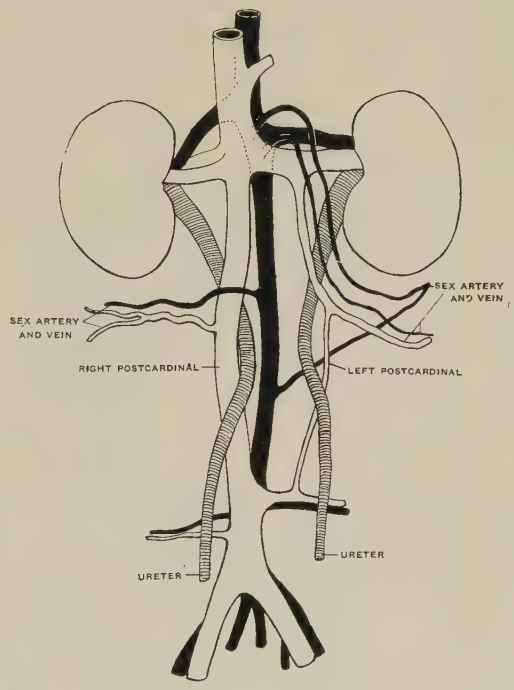
60 Marsupial Type of vena cava posterior. Vena cava posterior of Petrogale (spec.?). After McClure, figure VI, '03.

61 Adult cat (Darrach Series). Persistence of cardinal collateral channel (*C.C.*, fig. 1), in conjunction with right and left posterior cardinal veins. A combination of *Type* AD and the *Marsupial Type* of vena cava.

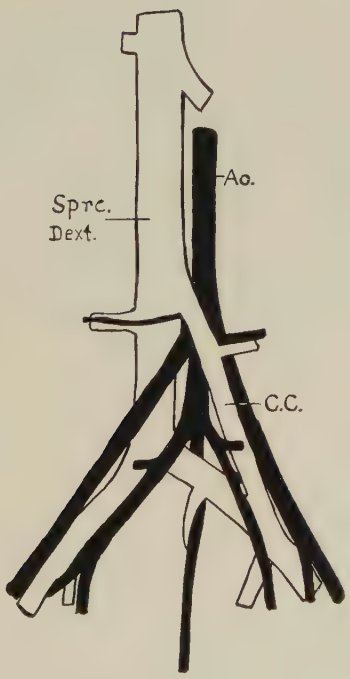
62 Adult cat (after McClure, fig. 4, '00). Persistence of cardinal collateral channel (*C.C.*) and right supracardinal vein (*Sprc.Dext.*, fig. 1). Princeton Morphological Museum, no. 603.


Aorta
V.Cava (C.C.)

60


SEX ARTERY AND VEIN
SEX ARTERY AND VEIN
RIGHT POSTCARDINAL
LEFT POSTCARDINAL
URETER
URETER

61

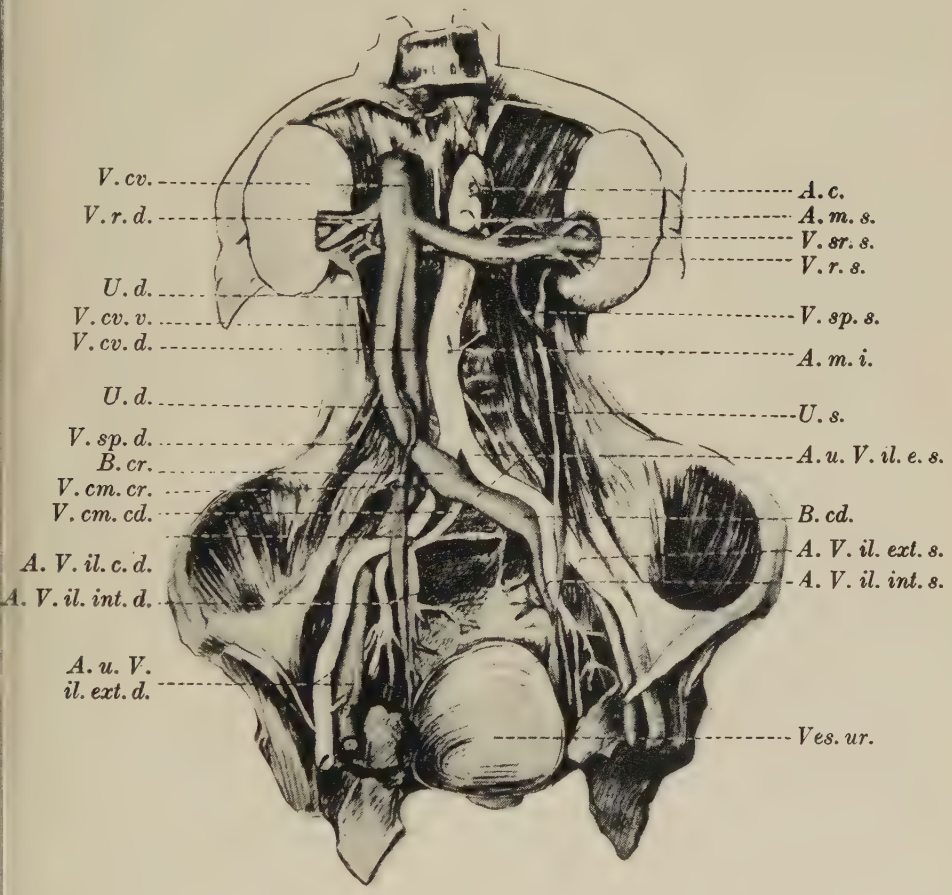

Ao.
Spre.
Dext.
C.C.

62

PLATE XLVI

EXPLANATION OF FIGURE

63 Adult man (after Wicke, Abb. 1, '27). Persistence of cardinal collateral channel (*C.C.* in fig. 1), in conjunction with right posterior cardinal and right supracardinal veins. Periureteric and circumumbilical venous rings present on right side. Legends by Wicke.


V. cv.
V. r. d.
U. d.
V. cv. v.
V. cv. d.
U. d.
V. sp. d.
B. cr.
V. cm. cr.
V. cm. cd.
A. V. il. c. d.
A. V. il. int. d.
A. u. V. il. ext. d.
A. c.
A. m. s.
V. sr. s.
V. r. s.
V. sp. s.
A. m. i.
U. s.
A. u. V. il. e. s.
B. cd.
A. V. il. ext. s.
A. V. il. int. s.
Ves. ur.

63

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LIFE PROCESSES AND SIZE OF THE BODY AND ORGANS OF THE GRAY NORWAY RAT DURING TEN GENERATIONS IN CAPTIVITY

PART I. LIFE PROCESSES

HELEN DEAN KING

PART II. SIZE OF THE BODY AND ORGANS

HENRY H. DONALDSON

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

1929

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PART I. LIFE PROCESSES

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HENRY H. DONALDSON

The Wistar Institute of Anatomy and Biology

TWENTY-TWO CHARTS

PREFACE

When it was recognized that the mutant albino rat differed from the ancestral gray Norway not only by the absence of pigmentation, but also in the proportional development of its organs, an attempt was made to feralize the Albino. This was done by planting Albinos where they might lead a wild life, in order to see how far, under these conditions, they would return toward the ancestral type. Five such colonies were planted in localities ranging from the Ipswich sands in Massachusetts to the dry Tortugas in the Gulf of Mexico, but for various reasons all of them failed. This first effort to determine whether the albino rat would revert, under wild conditions, to the gray Norway type, having thus met difficulties that halted it, the problem was revised, and it was next considered whether captivity and the accompanying treatment applied to the wild gray Norway might reduce it to the organ constitution of the Albino. Observations along this line seemed worth making, as we have no scientific record for any of our domesticated animals which show how domestication has changed them from the wild types from which they came.

Further, speaking broadly, no such tests are now possible, except perhaps in one or two instances, since the wild types are either unknown or for some reason not available, and consequently the data for comparison can hardly be had. In the case of the Norway rat, the gray form is definitely wild, depending on its own wits and exertions for its existence, while the albino mutant of the laboratory has now lived for many generations as a captive animal and may be fairly grouped with the other domesticated animals associated with man.

Consideration at once shows that continued captivity might be expected to alter the organ composition of the gray Norway. Such being the case, the alterations which prove to be similar to those now found in the Albino could be credited to captivity and treatment, while other modifications in the Albino not represented in the captive Norway could be referred to albinism.

It was on the basis of the foregoing considerations that the breeding of the Norway rat in captivity was undertaken by Doctor King in 1919. Naturally, this is a very long-time study which will require years for its completion. For this reason, occasional reports of progress seem to be permissible, and the following pages constitute an initial report of the observations on the first ten generations of the Norway rat in captivity.

INTRODUCTION

The purpose of this study is to determine such changes as may occur in the life processes and in the body and organs of the gray Norway rat when bred for a number of generations in captivity. In the case of the Norway rat, both the wild gray race and the captive albino race are readily available for study. When these are compared, marked differences in behavior and in the size of several organs are found. We wish ultimately to determine, in the case of the Albino, how far these differences may be due to prolonged captivity and how far to the condition of albinism—or possibly to other

causes, such as unconscious selection—applied to the albino strain. As contributory to this end, the effects of captivity on the wild gray Norway have been selected for study.

It may be remarked that the term 'captivity' is here used for the conditions under which the caged rats have been kept. The term 'domestication' has previously been employed to describe these same conditions, but as this latter term has wider connotations, such as a modification of the animal to meet human needs—which is absent in this instance—the term 'captivity' has been preferred, implying, as it does, merely a restricted range of activity and feeding, rather than foraging.

A general statement of the differences between the two races is given in "The Rat" (Donaldson, '24, p. 383). The wild Norways are more excitable and much more savage. They gnaw their cages. The body weight is less for a given body length, hence it is a slighter animal. The skeleton is relatively heavier, also the suprarenals (both sexes) and the testes and ovaries. The thyroid is of like weight, but the hypophysis distinctly lighter, in both sexes—this last difference being most marked in the female. On the other hand, the brain and the spinal cord are both heavier than in the Albino. The special relations will be noted as they come up for discussion.

It should be kept in mind that the present report applies only to the first ten generations of captive gray Norways, and is, therefore, a preliminary and tentative statement of results, since these data do not afford a basis for final conclusions. The observations are being continued and at present the nineteenth generation of these captive Grays has been obtained.

The selection of the first ten generations for treatment as a group depends on the fact that in the eleventh generation the strain 'broke' and several mutant varieties appeared, so that these ten generations are marked off by this event from those that follow.

In this paper the observations on the life processes are discussed in part I by King and those on the changes in the size and weight of organs and of the bones in part II by Donaldson.

Preceding these descriptions a statement is made concerning the conditions in the colony during the period under consideration. These conditions have an important bearing on the changes that have been observed.

The general care of the rats was not equally good during the period of seven years (1919–1926) which elapsed before the tenth generation was examined. In order to get at any effects of colony conditions on the captive Grays, the time relations of important events to the time relations of the several generations have been plotted in chart 1. In this chart each generation is represented by a vertical line running between the average date of birth, above, and the average date of killing, below. In individual cases, of course, birth preceded and killing followed the average date, but it was thought that this method of presentation would answer the present purpose of showing to what generations any particularly unfavorable conditions applied.

In the middle of 1922, the rats became infected with lice, and in September, 1922, were twice treated with larkspur solution for disinfection. This is a severe ordeal and, as the chart shows, affected younger animals in G_5 and G_6 and prospective mothers in G_7.[1] In the summer of 1923, great heat prevailed for a time in the colony and could have affected most readily G_6, G_7, G_8, as these individuals were at this time only half grown or younger.

From the middle of 1923 to the latter part of 1925, the care of the animals was not satisfactory, the caretaker being inefficient.

The observations to be reported show retardations in growth which apparently coincide with the unfavorable conditions represented by the louse infection, the larkspur treat-

[1] The successive generations are designated by G with a subscript giving the number of the generation in the series.

ment, and the excessive temperature of 1923. The effects of inadequate general care, as indicated on the chart, may also have been a factor contributing to the reduced body weights shown by the several generations from G_5 to G_8.

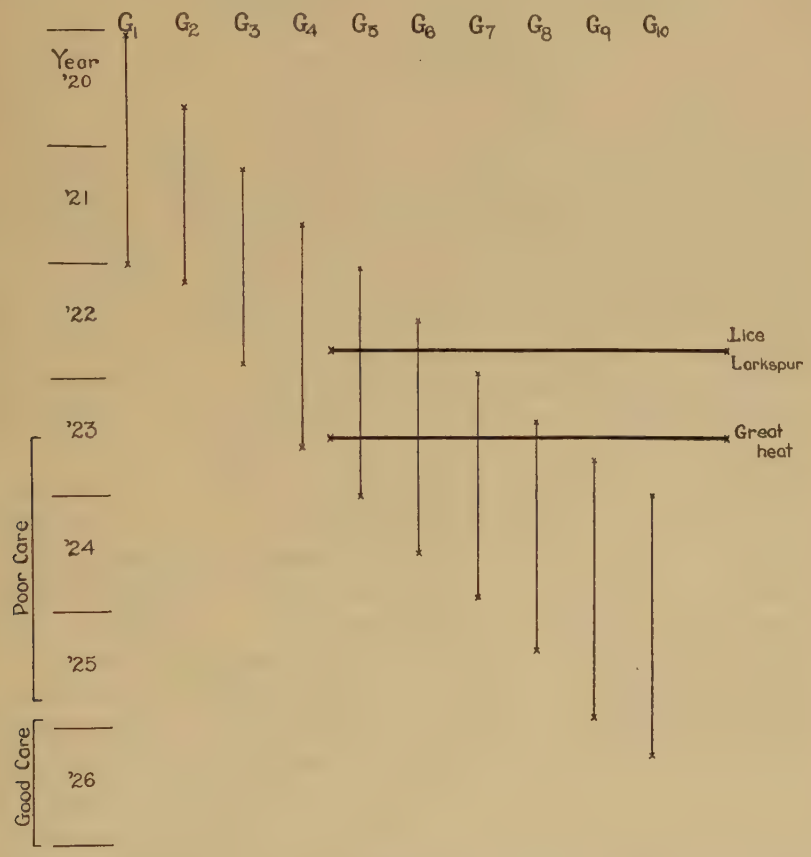

Chart 1 Showing birth date and killing date for each generation of captive Grays and indicating the incidence of unfavorable conditions.

PART I. LIFE PROCESSES

HELEN DEAN KING

MATERIAL AND METHOD

In the spring of 1919, thirty-six wild Norway rats were brought into The Wistar Institute Animal Colony to be used in starting a strain of captive Grays. These animals, comprising sixteen males and twenty females, had been trapped in various localities in the outskirts of Philadelphia, and all of them appeared to be vigorous and healthy. The smallest individuals were apparently about three months old at the time they were captured; the largest were adults at least a year old. In spite of the changed conditions of environment and of nutrition to which these rats were subjected, most of them lived for many months and some attained a body weight of over 400 grams.

Only six of the wild females cast young during the period of their captivity, as far as is known. From these females a total of twenty-one litters, containing 139 young (69 males and 70 females), was obtained. When breeding had ended, parents of litters were killed, measured, and carefully dissected; various organs were weighed, and certain bone measurements were taken. The findings were found to agree, in all essential respects, with those for a large series of wild Grays that Doctor Donaldson had examined previously. It is a fact of importance that this experiment was started with animals that were normal in every respect after months of caging, since it indicates that life in captivity for this period did not produce any marked changes in body structure. To what extent body functions were altered could not be determined, since practically nothing is known of life processes in wild Norways.

Wild gray females living in captivity show a pronounced tendency to destroy their young at birth, as Miller ('11) has noted. Casualties were so frequent with the litters cast by wild females in our colony that it was evident it would be impossible to obtain the animals needed to start a strain of captive Grays if the mothers were depended upon to rear their offspring. This difficulty was solved by removing the young rats from the nest as soon as they were discovered and giving them to a lactating albino female to rear. In a few cases albino females refused to suckle the substitutes for their own offspring, but in the majority of cases they accepted the young gray rats and reared them successfully. A total of 106 gray rats of the first generation, 51 males and 55 females, were brought to maturity and used for breeding stock.

That the early postnatal growth of gray rats that were suckled by albino foster mothers was not retarded is shown by a test made with a litter cast by a wild female that became relatively tame and did not greatly resent the temporary removal of her young from the nest. Four of the eight young in this litter were given to an albino female to rear; the other four young remained with the mother. At thirteen days of age the young suckled by the albino female weighed, on the average, 3 grams more than the other members of the litter. At thirty days of age, when all the rats were weaned, the average weight of those reared by the albino female was nearly 4 grams greater than that of the individuals their mother suckled. In later life, individuals of the same sex showed no pronounced differences in body weights.

Females of the first generation born in captivity did not show such a marked tendency to destroy their young as did their wild mothers. If the nest was disturbed very little when the young were removed for examination, they could be returned with a fair chance for life and future care. Individuals in the second and in all subsequent generations were reared by their own mothers.

In each generation about fifty individuals of each sex were reared for breeding and for a study of various life processes. The litters selected for this purpose contained an equal, or nearly equal, division of the sexes, and they were always of medium size (four to seven young) in order to avoid the possible effects of extreme litter size on body growth. Mortality among gray rats is often very heavy during the first few weeks of postnatal life, due to a variety of causes. In each generation, therefore, more litters were reared than the requirements of the experiment demanded, and those were discarded in which any individual died before reaching sixty days of age. Such a method of selecting breeding stock automatically eliminated weak individuals and assured strong, vigorous animals as parents of the following generation.

All of the captive Grays in the first ten generations lived under the same conditions of housing and of nutrition. Their food was a well-balanced, cooked ration composed of various cereals, meat, and vegetables; raw food, such as lettuce, carrots, cabbage, and fruit, was given two or three times a week. Variations in temperature to which these animals were subjected were not great. The colony room is adequately heated in winter and is relatively cool in summer. The cages that housed these rats were very large and therefore gave plenty of opportunity for exercise. The only unfavorable conditions affecting these rats were those referred to in the introduction.

All rats in the litters saved for breeding were weighed periodically. Birth weights were recorded if the young were obtained before they had suckled, otherwise the first weighing came at the age of thirteen days. The rats were weighed again when weaned, at thirty days of age, and thereafter at intervals of one month until they attained the age of twenty months. At this time a definite number of males was given to Doctor Donaldson for dissection, together with any females that had not cast a litter for at least two months. Females that were still breeding were kept for a longer time, in order that the litter records might cover the entire reproductive period.

BODY WEIGHT AT BIRTH

In the early generations of captive Grays parturition seemed to occur at night or in the early morning more often than during the working hours of the day; consequently, only a small number of litters were obtained before the individuals had suckled. Birth weights were taken for the twenty-six individuals in four litters cast by wild females. The twelve males in these litters had an average weight at birth of 5.34 grams; the average weight for the fourteen females was 5.09 grams.

In the first ten generations of these rats individual birth weights were recorded for a total of fifty-six litters, containing 177 males and 143 females. The average weights of the males varied from 5.07 to 5.82 grams in different generations; those of the females from 4.23 to 5.68 grams. There was no definite trend to the birth weights, either individual or average, as the generations advanced. In many cases individual females had a heavier weight at birth than one or more of the males in the same litter, but in each generation the average weight of the males exceeded that of the females. In fourteen litters of the tenth generation the forty-three males weighed, on the average, 5.29 grams at birth; the thirty-eight females had an average weight of 5.03 grams. These weights do not differ significantly from those of individuals in the first generation.

No other determination of the birth weights of gray Norway rats has been recorded, except that of Miller ('11), who states that the offspring of wild females in captivity weigh, on the average, 6.4 grams at birth, the males tending to be slightly heavier than the females. The number of individuals on which this determination is based is not given.

Apparently the weight of the gray rat at birth tends to be somewhat heavier than that of the albino rat. The findings of Jackson ('13) and of King ('15), for various series of Albinos, show that in this race the range of variation in the average birth weights of males is from 4.6 to 5.1 grams; that of females being from 4.5 to 4.9 grams. In both the

gray and the albino race of Norways, however, the average birth weight of the males, as a rule, exceeds that of the females.

Birth weight in the rat is influenced by a number of different factors, such as the age and physical condition of the mother, the size of the litter and its position in the litter series, and the length of the gestation period (King, '15). Environmental and physiological factors, therefore, may be largely responsible for the differences in the birth weights of gray and of albino young. To decide this point definitely will necessitate comparison between the birth weights of offspring from a large number of gray and albino females of various ages, and from litters of the same size. The collection of data for this purpose has been in progress for some years.

GROWTH IN BODY WEIGHT

There are two distinct phases of body growth in mammals, each of which consists of several growth cycles which merge into each other at transitional periods. The first of these phases comprises the period between conception and puberty, and in it the time rate of growth increases with the increase in body size. The second phase covers body growth during adult life, and here the time rate of growth decreases while the size of the organism increases (Brody, '26).

Our knowledge of the initial stage of the first growth phase in the rat, that occurring during fetal life, is based on the observations of Stotsenburg ('15), who studied the weight increase in albino fetuses from the thirteenth to the twenty-first day of gestation. During this period the percentage rate of growth per day was found to be approximately 53 per cent.

No study has been made, as yet, of embryonic growth in the gray rat. Data for the postnatal growth in individuals of the first generation of these captive Grays were published some years ago (King, '23), and they are here reproduced in tables 4 and 5. Points of interest in connection with these data will be discussed when comparison is made between them and the findings in later generations.

Although the males in the first generation of captive Grays were heavier than the females at birth, the latter grew more rapidly during early postnatal life. At thirteen days of age the average weight of the females exceeded that of the males by 0.9 gram, and was 3.6 grams greater at thirty days. The weight relation of the sexes was reversed, however, when the animals were sixty days old, and at all subsequent ages males were much heavier than females.

In his first study of body growth in the albino rat, Donaldson ('06) stated that males were heavier than females at birth and up to seven days of postnatal life, when a period of more active growth on the part of the females began. Growth graphs, constructed from data for the two sexes taken at intervals of one or two days, cross at the fourteen-day period, and that for the females runs above the one for the males until the sixty-day period. At this point the graphs recross, and that for the males runs at a higher level until its end. There is, therefore, a very striking similarity between the growth relations of the sexes in the strain of Albinos studied by Donaldson and those of individuals belonging in the first generation of captive Grays. Like relations were not found, however, in several subsequent studies on the growth of the albino rat (Jackson, '13; King, '15 a; Donaldson, '24), nor do they appear in the findings for the later generations of gray rats. In all of these cases the males have a heavier body weight than the females throughout the entire life span.

It seems probable that the differences in the weight relations of the sexes in these various series of Norway rats were due to environmental and nutritive conditions, not to factors inherent in the strains. At the time that Donaldson began his studies the rat was not as commonly found in biological laboratories as it is to-day, and little was known regarding the conditions necessary to maintain the animals in health and to enable them to grow and reproduce at a normal rate. Donaldson's rats were fed chiefly on milk-soaked bread and on corn, with the occasional addition of a little meat. Nutrition studies of later years (Osborne and Mendel, '16, '16 a,

'18, etc.; Slonaker and Card, '23; Evans and Bishop, '22, '22 a, '23; Kennedy, '26) have shown conclusively that rats require a well-balanced, highly nutritious diet for normal growth and for reproduction and that these animals respond very quickly to changes in nutritional regime. Males, seemingly, are more influenced by unfavorable conditions than are females, especially during fetal and early postnatal life, when the mortality among them is much greater (King, '21). It seems probable, therefore, that the slow growth of the males during early postnatal life in Donaldson's first series of Albinos was due to inadequate nutrition.

The growth relation of the sexes during early life in the first generation of captive Grays cannot be attributed to inadequate nourishment, since these animals received an abundance of highly nutritious food. Moreover, in the various strains of albino rats in the colony on the same nutritional regime, the growth of the males was more rapid than that of the females at all age periods. It must be assumed, therefore, either that the growth relations of the sexes during early postnatal life in the first generation of captive Grays was that normal for the race in its wild state or that lack of adjustment to conditions of life in captivity adversely affected the wild mothers of these rats and, through her, influenced the vigor of the young, particularly that of the males, so that they did not grow at a normal rate. Whichever of these alternatives one chooses to accept, it is certain that after one generation in captivity gray rats became so well adjusted to their new habitat that the course of body growth in their offspring showed the same sex relations at all age periods as those usually found in albino rats.

No marked changes occurred in the growth of the individuals in any of the first ten generations of captive Grays that made one generation stand out conspicuously from the rest. It seemed advisable, therefore, to combine the data for each three succeeding generations, beginning with the second, thus making three groups of data for nine generations. Data for the first generation stand alone, to be used

for comparison with the findings in later generations to indicate the changes in the rate and in the extent of body growth that resulted from life in captivity.

Weight changes in the body with age for individuals in the first group, comprising the second to the fourth generations, inclusive, are shown in table 1.

TABLE 1

Showing the increase in the weight of the body with age for individuals in the second to the fourth generations of captive gray rats

AGE IN DAYS	MALES				FEMALES			
	Number of individuals	Body weight in grams			Number of individuals	Body weight in grams		
		Average	Highest	Lowest		Average	Highest	Lowest
13	161	17	27	12	170	16	24	12
30	161	41	67	30	170	42	59	29
60	161	98	188	54	170	86	148	46
90	160	139	255	68	170	117	202	73
120	160	182	317	92	164	143	220	87
151	160	209	384	102	167	160	245	92
182	160	233	382	122	164	174	268	108
212	160	256	410	130	167	190	300	110
243	160	273	458	138	165	202	295	114
273	159	285	480	146	164	210	384	113
304	157	296	517	163	160	219	422	137
334	157	306	510	172	156	225	381	151
365	151	316	538	188	156	235	404	156
395	145	328	535	192	155	243	406	140
425	141	337	615	210	151	250	439	164
455	137	345	604	221	143	255	400	166
486	130	351	589	209	135	259	412	157
516	117	357	577	216	130	263	433	163
547	111	362	572	223	124	268	428	176
578	98	375	566	237	103	271	410	180
608	89	374	592	225	90	283	430	171

In the first group, as indicated by corresponding entries for the two sexes in table 1, the average weight of the males at thirteen days of age was only slightly greater than that of the females. At later age periods the excess in favor of the males increased greatly.

Although the average age at which the females of this group began breeding was 252 days (table 6), it is probable that the great majority of them had become sexually mature by the time they were six months old. At this age, which marks approximately the merging of the first growth phase into the second, the average body weight of the males was 59 grams greater than that of the females. This difference is 25.3 per cent, if the body weight of the males is taken as the standard in computation. In adult life both males and females became increasingly heavier, but the percentage differences in their weights changed but little. The maximum difference, 27.7 per cent, was reached when the rats were 578 days old.

As shown in table 1, no female in the first group attained a maximum weight exceeding that of a male of the same age, nor was the lowest weight recorded for any male below that for a female of like age.

Growth data are given in table 2 for the second group of captive Grays, comprising individuals in the fifth to the seventh generations, inclusive.

Differences between the average body weights of males and females during early postnatal life were about the same in the second group as in the first one (compare table 1 and table 2). At 182 days, however, males in the second group exceeded the females in body weight by only 47 grams, or 19.6 per cent. The maximum male excess in weight, 21.6 per cent, came at the age period of 578 days.

Maximum body weights for both sexes were higher in the second group than were those in the first group, and the average weights were greater at all age periods.

Data for the third group, including individuals in the eighth to the tenth generations, inclusive, are shown in table 3.

In the third group, as in the two preceding ones, sex differences in the average body weights were small during the early growth period, and increased as the animals grew older. The male excess in weight at 182 days was 42 grams (17.4 per cent), and its maximum (21.3 per cent) came at 578 days, as shown in table 3.

In each of the generation groups the percentage differences in the average body weights of males and females remained fairly constant throughout adult life, the variation being but 2 per cent in the first two groups and 4 per cent in the third group. These differences tended to decrease, however, as the

TABLE 2

Showing the increase in the weight of the body with age for individuals in the fifth to the seventh generations of captive gray rats

AGE IN DAYS	MALES				FEMALES			
	Number of individuals	Body weight in grams			Number of individuals	Body weight in grams		
		Average	Highest	Lowest		Average	Highest	Lowest
13	157	19	27	12	159	18	26	14
30	157	45	74	27	159	44	74	28
60	157	102	195	63	159	87	140	48
90	157	148	273	67	158	120	188	60
120	157	187	310	95	148	149	248	95
151	157	217	375	104	152	176	281	114
182	157	240	433	126	151	193	319	123
212	157	262	428	157	153	213	337	128
243	156	282	434	166	152	227	334	137
273	155	298	466	180	146	240	359	143
304	155	311	474	188	149	249	392	153
334	154	320	496	211	147	252	382	158
365	153	332	544	224	150	263	402	156
395	152	341	568	236	147	269	425	162
425	148	350	575	243	142	275	431	165
455	145	358	608	246	142	283	437	168
486	141	359	647	256	136	285	455	174
516	134	366	636	264	129	289	415	188
547	128	373	639	270	127	293	445	185
578	120	381	637	260	117	299	474	185
608	113	379	620	221	109	300	459	168

generations advanced. The male excess in weight in the third group was 7.9 per cent lower than that in the first group when the animals were 182 days of age, and 6.4 per cent less when the maximum was reached at 578 days. This progressive lessening in the percentage differences between the body weights of males and females was due, as a comparison of the

data in tables 1 to 3 shows, to the fact that body-weight increase during adult life tended to be greater in females than in males as the generations advanced. The increased weight of the females is to be attributed chiefly to the accumulation of fat in the body tissues, not to growth of the body as a

TABLE 3

Showing the increase in the weight of the body with age for individuals in the eighth to the tenth generations of captive gray rats

AGE IN DAYS	MALES				FEMALES			
	Number of individuals	Body weight in grams			Number of individuals	Body weight in grams		
		Average	Highest	Lowest		Average	Highest	Lowest
13	156	19	32	14	162	18	32	15
30	156	48	72	32	162	46	72	30
60	156	99	190	59	162	88	143	52
90	156	145	243	79	162	122	183	73
120	156	193	297	117	160	154	255	103
151	156	219	332	135	159	181	286	110
182	156	241	364	156	156	199	294	124
212	156	259	395	183	153	208	305	137
243	156	274	406	196	150	223	322	142
273	155	286	428	212	158	231	350	146
304	153	296	430	224	157	237	338	149
334	153	304	464	233	155	246	384	154
365	150	315	480	244	152	254	391	163
395	148	320	490	244	153	261	404	174
425	145	332	508	257	147	270	408	187
455	144	340	506	275	148	273	403	184
486	141	346	485	276	145	278	412	217
516	133	355	502	265	147	281	409	217
547	123	366	489	277	147	289	423	219
578	113	375	497	224	140	295	408	205
608	109	378	538	282	130	298	436	192

whole, since body length in females of the later generations was no greater than that in females of the earlier generations (table 12). Body growth of males during the second growth phase was apparently less influenced by the favorable environmental and nutritive conditions under which the animals lived than was that of females.

Since a graphic representation of the data will bring out more clearly the changes in body growth with age in successive generations of these rats, graphs for the growth of the males, constructed from data in tables 1 to 3, are shown in chart 2.

All three graphs in chart 2 run close together throughout their entire course, especially so at the beginning, where they have been separated somewhat more than the data warrant in order that individual graphs can be clearly followed.

The most important fact brought out in chart 2 is that body growth in captive gray males is accelerated during the period preceding puberty as the generations advance. The graph for the second group (fifth to seventh generations) runs slightly higher than that for the first group (second to fourth generations) from its beginning. The graph for the third group (eighth to tenth generations) crosses that for the second group at the 120-day period and then runs higher than either of the other graphs up to the 212-day period, subsequently falling to the level of that for the first group. The fact that males of the last group did not attain as heavy a weight in adult life as did those of the second group is not significant, since the growth of these rats during adult life was probably affected somewhat by unfavorable conditions in the colony (chart 1).

Growth graphs for the females in the three generation groups are shown in chart 3. These graphs were constructed from the data in tables 1 to 3.

Growth graphs for females (chart 3) differ from those males (chart 2) in two respects: 1) they are more widely separated throughout the greater part of their course; 2) all of them run at a lower level, since gray females are, on the average, much smaller than males at all age periods after weaning.

The graphs in chart 3 run very close together up to the 100-day period, when they begin to diverge. A progressive change in the form of the graphs as the generations advanced is not as clearly marked as in the case of the males. The

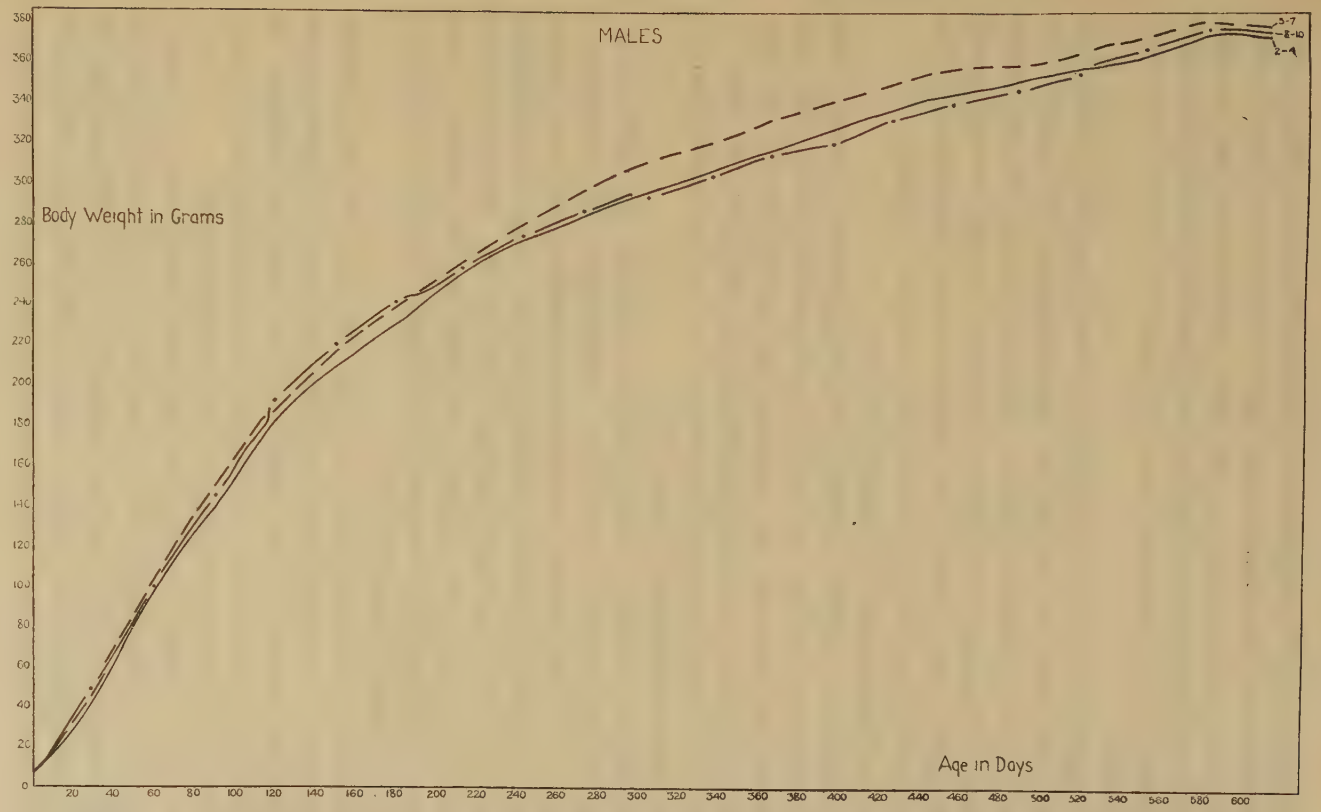

Chart 2 Graphs showing the increase in the weight of the body with age in successive generation groups of captive gray males.

graph for the third group of females, however, runs higher than either of the other graphs up to the 200-day period, thus indicating that females in the later generations had a relatively greater acceleration in body growth during the early portion of the life span than did females in the earlier generations.

Females in the later generations were heavier in adult life than were those in the earlier generations, as the position of the graphs in chart 3 shows. That the graph for the third group of females runs lower than that for the second group during the latter part of its course is due, doubtless, to the same cause as that advanced for a similar relation in corresponding graphs for males (chart 2).

In order to show the change in body growth of males that had occurred at the end of ten generations, data for body weights at different age periods for individuals in the first and for those in the tenth generation are given in table 4. The data for the first generation are reproduced from a previous publication (King, '23).

Data for average body weights, as given in table 4, are shown graphically in chart 4. This chart also contains a graph (3) for a series of stock albino males that were reared under similar conditions of housing as the gray rats, although they were fed on 'table scraps,' not on the ration that for some years has been given to all rats in the colony. Data from which graph 3 was constructed were given in a previous paper (King, '15 a, table 3).

In chart 4 the graph (2) for gray males of the first generation runs at a much lower level throughout its entire course than the graph (2) for males of the tenth generation. It shows no pronounced curve at its beginning, but mounts steadily upward and tends to be a straight line.

Graph 2, that for males of the tenth generation, is also a nearly straight line from its beginning up to about the 120-day period, when it curves upward. At the 240-day period the graph begins to flatten, and subsequently it runs fairly straight until its end. The space between graph 1 and graph

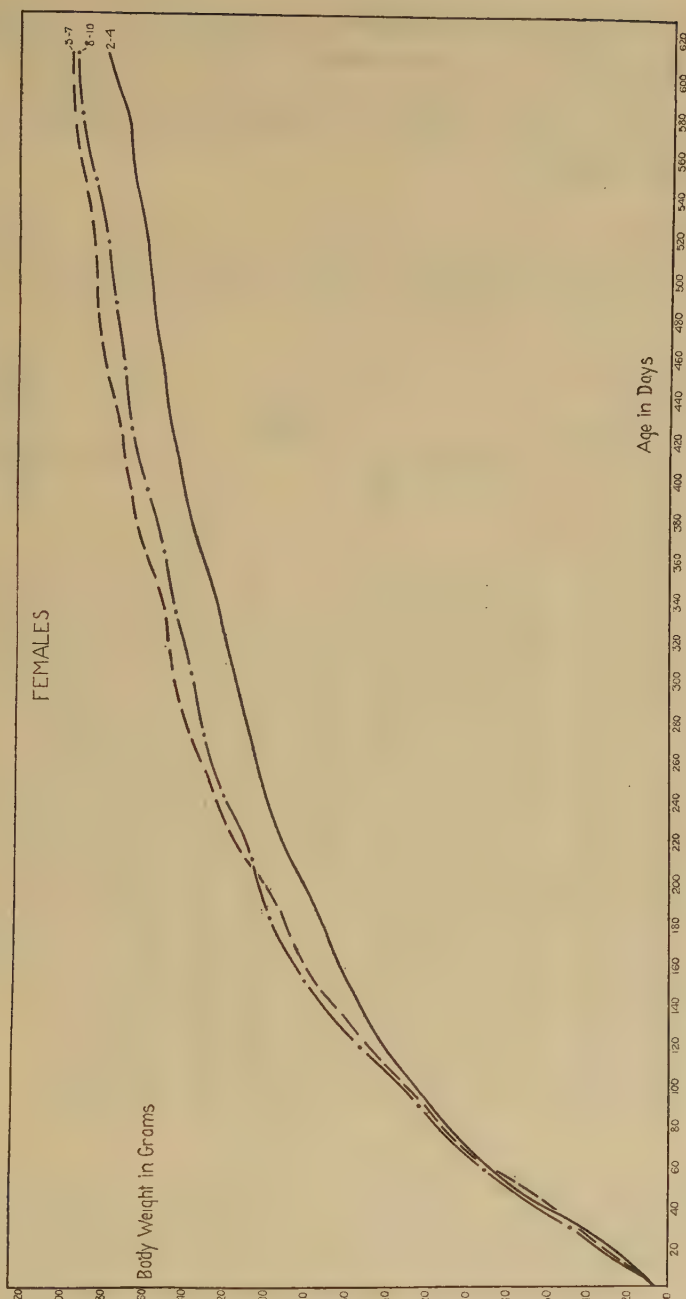

Chart 3 Graphs showing the increase in the weight of the body with age in successive generation groups of captive gray females.

2 at the 180-day period indicates a difference of 39 grams, or 19.9 per cent, in the body weights of these two series of males. At the end of the weighing period (608 days), the position of the graphs indicates a difference of 4.1 per cent in the body weights.

TABLE 4

Showing the increase in the weight of the body with age and the coefficients of variation for males in the first and in the tenth generation of captive gray rats, also coefficients for a series of stock albino males

AGE IN DAYS	FIRST GENERATION GRAY MALES					TENTH GENERATION GRAY MALES					STOCK ALBINO MALES
	Number of individuals	Body weight in grams			Coefficients of variation	Number of individuals	Body weight in grams			Coefficients of variation	Coefficients of variation
		Average	Highest	Lowest			Average	Highest	Lowest		
13	51	15.4	21	12	12.2±0.82	50	20	32	16	15.3±1.03	11.8±0.79
30	51	33.4	41	26	13.0±0.86	50	49	72	37	16.5±1.11	10.2±0.68
60	51	81.9	144	49	24.1±1.60	50	92	161	59	24.3±1.66	17.0±1.14
90	51	115.9	201	68	26.2±1.74	50	137	243	79	29.3±1.98	14.8±0.99
120	51	148.6	245	70	28.2±1.88	50	180	277	126	22.6±1.53	13.4±0.90
151	51	176.1	284	87	24.2±1.61	50	217	322	155	17.7±1.19	13.3±0.89
182	51	195.9	300	110	23.4±1.55	50	235	364	177	16.5±1.11	14.2±1.22
212	51	218.4	330	128	21.8±1.45	50	254	368	193	15.7±1.06	14.0±0.96
243	50	235.4	374	140	23.7±1.60	50	268	398	209	14.2±0.95	13.9±0.99
273	50	253.5	379	154	22.4±1.50	49	282	404	218	13.8±0.94	13.4±0.99
304	50	265.5	380	156	21.5±1.45	49	290	410	230	12.1±0.82	14.0±1.11
334	50	276.7	395	170	19.8±1.33	49	301	464	233	13.3±0.91	13.7±1.13
365	48	287.3	428	184	19.5±1.33	48	312	480	246	13.2±0.91	13.0±1.16
395	48	297.4	442	185	19.5±1.34	48	318	490	244	14.3±0.98	12.6±1.22
425	47	312.2	457	200	17.6±1.22	48	327	508	263	14.0±0.97	13.4±1.32
455	47	322.3	471	188	19.2±1.32	48	335	506	277	13.1±0.89	13.6±1.67
486	46	332.0	459	226	17.3±1.21	47	346	485	279	12.1±0.84	15.0±2.06
516	43	335.0	476	226	17.4±1.26	44	354	502	284	12.2±0.87	
547	41	345.6	486	231	16.7±1.24	41	368	478	284	12.1±0.90	
578	39	358.0	516	233	18.3±1.39	37	374	497	277	12.2±0.95	
608	38	361.8	518	231	17.1±1.32	34	377	538	303	14.5±1.18	

The graph (3) for stock albino males has a distinctly different form from that of either of the graphs for gray males. At the age period from 40 to 150 days the graph has a very pronounced upward curve. Subsequently it flattens, and runs

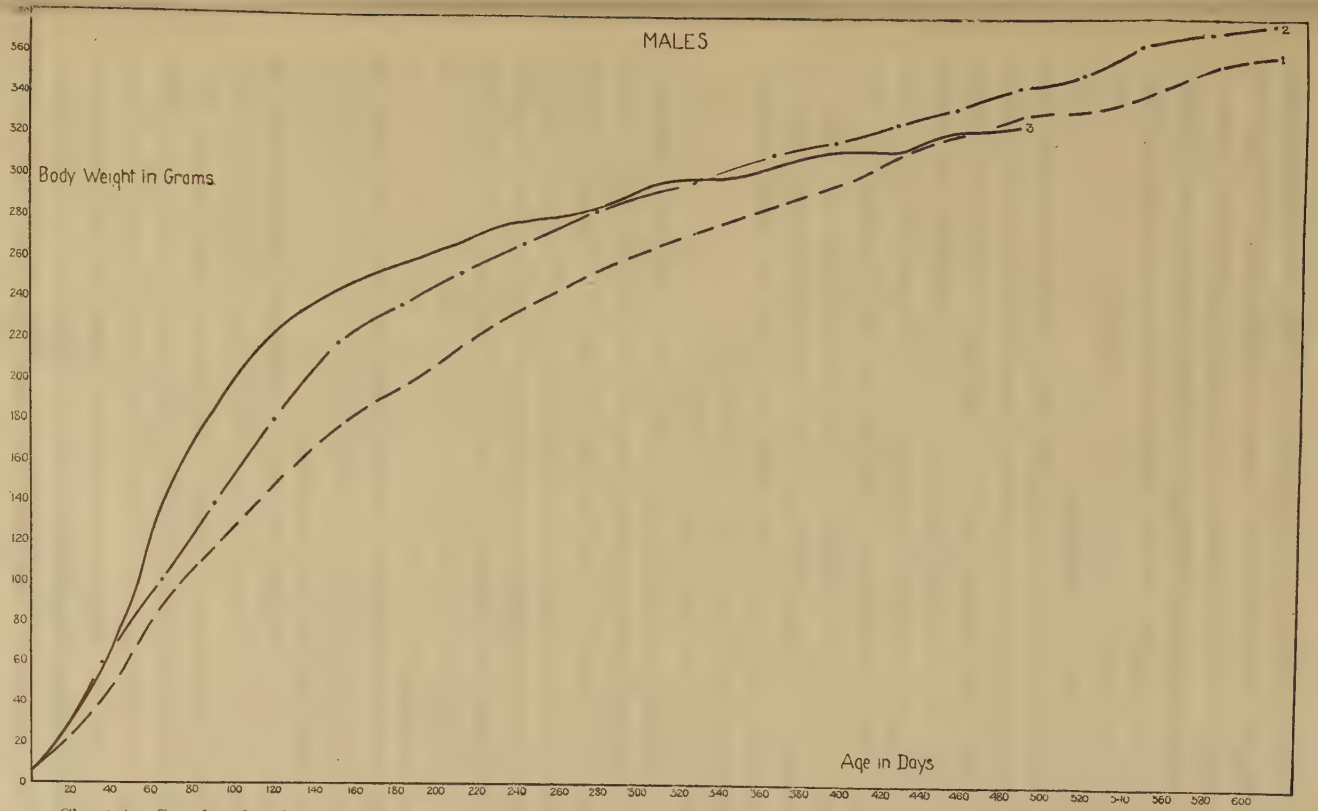

Chart 4 Graphs showing the increase in the weight of the body with age in series of male rats.
1. Graph for males in the first generation of captive Grays.
2. Graph for males in the tenth generation of captive Grays.
3. Graph for stock albino males.

nearly level until its termination at the 480-day period. Graphs might be constructed from available data for various other series of albino males that would, doubtless, have a slightly different course than that of the graph given in chart 4, but all such graphs would indicate very rapid growth during early postnatal life, which is, seemingly, characteristic for the albino race in general.

The relative positions of the graphs in chart 4 indicate that during the period of rapid growth, from 40 to 150 days, albino males were very much heavier than gray males of either series. At the 150-day period the space between the graph (3) for albino males and the graph (1) for gray males of the first generation represents a difference of 69 grams, or 28.2 per cent, if the body weight of the albino males is taken as the standard in computation. At this age period, however, albino males were only 28 grams, or 11.4 per cent, heavier than gray males of the tenth generation.

From the above analysis of data it is evident that the course of body growth in gray males had changed considerably at the end of ten generations of life in captivity. During the first growth phase the rate of growth was accelerated and was approaching the albino type. During the second growth phase the change was relatively slight, since the ultimate body weight attained by males of the tenth generation was only about 4 per cent greater than that of the males in the first generation.

Data for the increase in the weight of the body with age for females in the first and for those in the tenth generation of captive Grays are given in table 5.

In table 5 the findings for gray females parallel those for the corresponding series of gray males (table 4) in that they show that females of the tenth generation were heavier than females of the first generation at all age periods for which records were taken.

Chart 5 shows graphs constructed from the average body weights of gray females as given in table 5. In this chart, also, is a graph (3) for the body growth of albino females,

litter mates of the males whose body weights form the basis of graph 3 in chart 4.

The relative position and general trend of the graphs in chart 5 are much the same as those in chart 4, but all of them

TABLE 5

Showing the increase in the weight of the body with age and the coefficients of variation for females in the first and in the tenth generation of captive gray rats, also coefficients for a series of stock albino females

AGE IN DAYS	FIRST GENERATION GRAY FEMALES					TENTH GENERATION GRAY FEMALES					STOCK ALBINO FEMALES
	Number of individuals	Body weight in grams			Coefficients of variation	Number of individuals	Body weight in grams			Coefficients of variation	Coefficients of variation
		Average	Highest	Lowest			Average	Highest	Lowest		
13	59	16.3	21	12	14.6±0.90	52	19	32	17	13.1±0.90	11.4±0.76
30	59	37.0	45	27	17.3±1.07	52	47	70	35	14.1±0.93	11.0±0.74
60	59	75.2	110	47	19.8±1.22	52	83	121	52	19.6±1.09	15.7±1.05
90	59	100.5	152	70	16.9±1.04	52	117	183	73	23.8±1.58	12.5±0.95
120	59	122.6	184	77	16.0±0.99	52	156	255	114	20.1±1.33	10.3±0.75
151	56	142.0	201	98	16.6±1.03	49	186	276	128	16.5±1.13	10.4±0.73
182	54	153.5	256	111	17.8±1.13	50	203	277	162	13.9±0.94	12.3±0.90
212	50	172.1	272	117	17.9±1.20	50	212	298	164	14.1±0.95	12.4±0.91
243	52	189.3	278	133	18.2±1.26	46	225	318	173	13.9±0.98	12.6±0.91
273	51	204.2	327	131	19.1±1.27	50	234	363	186	17.0±1.15	11.5±0.89
304	47	209.8	301	123	19.9±1.38	51	239	330	184	15.4±1.03	10.3±0.79
334	49	221.5	345	128	20.6±1.40	50	247	360	181	16.4±1.10	10.8±0.87
365	47	232.8	344	152	20.4±1.41	48	256	374	203	16.9±1.16	10.7±0.91
395	47	242.2	383	135	22.3±1.54	49	258	388	188	17.3±1.20	11.5±0.98
425	43	247.5	380	156	21.1±1.47	46	266	368	199	15.7±1.10	10.9±0.94
455	44	260.5	359	172	18.9±1.35	48	268	385	209	15.2±1.04	8.9±0.99
486	43	257.9	382	182	19.5±1.41	47	272	392	217	14.1±0.98	13.4±1.77
516	37	264.1	373	163	18.3±1.43	48	275	400	219	14.8±1.02	
547	36	261.5	375	173	19.4±1.54	48	284	398	221	15.2±1.04	
578	34	266.5	360	168	16.4±1.34	46	291	403	224	16.0±1.12	
608	31	271.9	339	184	15.3±1.28	42	304	411	237	16.3±1.20	

run at a lower level, since in both races of Norways the females, as a rule, are not as heavy as the males at any age period.

In chart 5 the graph (1) for gray females of the first generation runs below either of the other graphs and tends to be

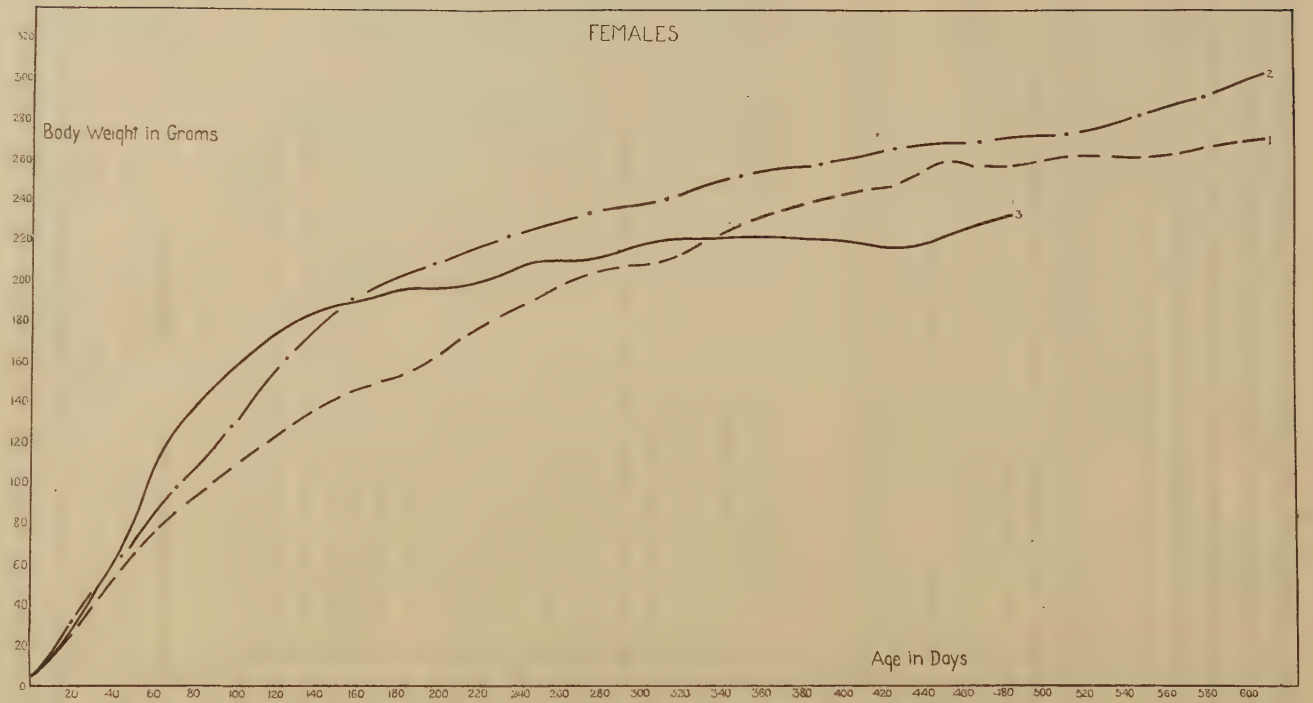

Chart 5 Graphs showing the increase in the weight of the body with age in series of female rats.

1. Graph for females in the first generation of captive Grays.
2. Graph for females in the tenth generation of captive Grays.
3. Graph for stock albino females.

a straight line, as these females showed no pronounced acceleration in body growth during early postnatal life. Graph 2, for gray females of the tenth generation, runs higher than graph 1 from its beginning until the end of its course. At the 150-day period the space between these graphs represents a difference of 44 grams, or 31 per cent, in average body weights. Subsequently these graphs run much closer, and their position at the end of the weighing period indicates a difference of but 8.1 per cent in the average body weights.

Albino females show the same marked acceleration in growth during the first growth phase as do albino males. In chart 5, as in chart 4, the graph (3) for Albinos runs much higher during the early part of its course than either of the graphs for Grays. At a later period, however, graph 3 flattens and falls below the level of the other graphs, since the albino females were not as heavy as gray females during adult life.

At 150 days of age albino females had an average body weight that was 44 grams, or 23.6 per cent, heavier than that of gray females of the first generation. At this age, however, gray females of the tenth generation had the same weight as that of the albino females, and they were much heavier in later life (chart 5).

The change in the rate and in the extent of body growth that had occurred in gray females at the end of ten generations of life in captivity was similar to that in the males. The trend was toward the type characteristic for albino rats in general. In only one series of Albinos for which data are available, that of Greenman and Duhring ('23), does the body weight of adult animals equal that of the tenth generation of captive Grays. These Albinos, however, were given exercise and were especially well cared for and fed.

The findings in these captive Grays are in accord with those for various domesticated animals, such as cattle, sheep, horses, etc., which are much larger at all age periods than their wild prototypes.

In the rat, as in the mouse and many other mammals, the period of most rapid growth ends at puberty, or very shortly afterward. The age at which sexual maturity was attained by gray females was not determined in any case by the exact method of Long and Evans ('22). The average age at which females of the first generation cast their first litters was 266 days; that of females of the ninth generation was but 200 days. In these rats, therefore, the beginning of reproductive life was advanced two months in the course of nine generations. Whether the more rapid growth of animals in the later generations induced earlier maturity or whether both the increase in the rate of growth and earlier maturity depended upon other factors is not known.

Robertson ('16) states:

> The fact that the syntheses which constitute the growth of an organism are self-accelerated or autocatalyzed implies the existence of substances capable of acting as catalysers of growth. We know that many of the glands of internal secretion, anterior lobe of the pituitary, the thyroid, and thymus are capable of exerting important influences upon the processes of growth—influences the lack of which become apparent when one of the glands is removed.

It seems not improbable that the changes which life in captivity induced in the rate and in the extent of body growth in gray rats, and the effects of captivity upon breeding, were not related as cause and effect, but that both depended upon some alteration in the endocrine system which tended to speed up the growth processes and to induce earlier breeding as the generations advanced. It is interesting, in this connection, to note that the one endocrine gland in these rats most affected by life in captivity was the hypophysis which, as shown by Evans and Long ('22), is capable of influencing body growth in the rat. This gland is somewhat larger in both sexes of captive gray rats than it is in the wild form, and has tended to approach the albino type, as will be shown in a subsequent section (part II, p. 87).

It may be argued that the course of body growth in captive gray rats of the first generation is not representative

of the growth of wild rats in their natural habitat, and that conditions incident to captivity, the lack of exercise, changed nutrition, excessive fear, etc., greatly retarded early growth, which is probably very rapid in the wild form. On this assumption, the more rapid growth of individuals in the later generations would indicate merely that the animals had become somewhat adapted to their changed environment and were, therefore, resuming their normal rate of growth. Such an argument cannot be refuted at present, or probably at any time, since there seem to be insurmountable obstacles in the way of a scientific study of growth processes in wild Norway rats.

Aside from restricting activity somewhat, the conditions under which captive Grays lived would seem to be far more favorable to growth and to reproduction than are those to which wild rats are usually subjected. The element of fear could certainly not have influenced the growth of caged rats more than it must affect that of wild Norways which are constantly harassed by enemies of many kinds. The food given caged animals was more abundant and more nutritious than that wild rats obtain by foraging, and temperature conditions in the colony room were not as extreme as those in nature. Lack of exercise may possibly have influenced the later growth of caged Grays, but it could hardly have changed growth processes in young rats that spend the greater part of their time in the nest. Taking all of these various factors into consideration, it seems probable that the course of body growth in the first generation of captive Grays is fairly representative of that in the wild forms, and that it can be used legitimately to indicate the effect of captivity on the body growth of individuals in the later generations.

Since the albino rat is a mutant variety of the gray Norway, one may assume, perhaps, in light of the findings for captive Grays, that the rapid growth of Albinos during early life is a result of their long domestication. They have seemingly become completely adapted to life and conditions of captivity, and they have lost all fear of man, which is a dominant trait

in wild Norways. This lessening of nervous tension, and its far-reaching effects through the nervous system, may be a factor that influenced the course of body growth in gray rats of the later generations, since these animals were relatively very tame as compared with wild Norways.

VARIABILITY IN BODY WEIGHT

Since changes in the rate and in the extent of body growth in captive gray rats took place slowly, as shown in the previous section, it seemed probable that any change that occurred in body-weight variability was also a gradual one. Coefficients of variation for body weights at different age periods were computed, therefore, only for individuals in the first and for those in the tenth generations. A comparison of these two series of coefficients will indicate to what extent variability in body weight had changed at the end of ten generations.

Coefficients of variation, with their probable errors, for males in these two generations are given in table 4; those for females are shown in table 5. Each of these tables also contains a series of coefficients for body weights in the stock Albinos used for comparison with the Grays in the analysis of body growth. The coefficients for Grays of the first generation, as well as those for Albinos, are reproduced from previous publications (King, '15 a, '23). Grouped data had to be used in calculating the coefficients for the thirteen- and for the thirty-day periods, since only the average body weights for rats of each sex were recorded in the weighings of the litters at these ages. Coefficients for all other age periods are based on individual data.

Variability in body weight, as indicated by the size of the coefficients in table 4, had the same general trend in all three groups of males: it was relatively low in young animals, rose quickly to a maximum, and then fell gradually to a level that was maintained, with unimportant fluctuations, throughout adult life. At the various age periods, however, variability in body weight differed considerably in the three groups.

Gray males of the tenth generation tended to be somewhat more variable in body weight during early postnatal life than did males of the first generation, and maximum variability came at an earlier age (table 4). Since, however, differences between the maximum coefficients, and between corresponding coefficients for all age periods up to and including 120 days, are not statistically important, it appears that no significant change in body-weight variability had occurred during early life.

Variability in body weight during adult life had changed considerably at the end of ten generations. In males of the tenth generation variability decreased rapidly, after attaining its maximum at the ninety-day period, and subsequently was much less than that in males of the first generation. The size of the coefficient for the age period of 120 days indicates a drop of 6.7 ± 2.50 from the maximum; at age periods from 151 to 578 days differences between the corresponding coefficients for the two series are well over three times their probable errors. In a single instance such a difference would not be deemed of much statistical value, but the fact that this difference is found at fifteen consecutive age periods, covering about one-half of the life span of the individuals, adds considerably to its importance. The conclusion seems justified that gray males of the tenth generation were less variable in body weight during adult life than were males of the first generation.

A comparison of corresponding coefficients in table 4 indicates that gray males of the first generation were significantly more variable in body weight at all age periods from 60 to 395 days than were stock albino males. Gray males of the tenth generation showed a body-weight variability up to the age of 151 days that was greater than that in albino males of like ages, but variability in adult life was about the same in the two series. It is evident, therefore, that the trend of variability in captive gray males, as the generations advanced, was toward that found in stock albino males.

In gray females of the first generation variability in body weight did not reach its maximum at an early age, as in the other two series of females (table 5), but increased gradually until the 395-day period, after which it declined slightly. These females were more variable in body weight at all age periods, excepting at 90, 120, and 608 days, than females of the tenth generation, but differences between corresponding coefficients are small in most cases and therefore not significant. At the end of ten generations, variability in body weight had decreased but little in gray females. Comparison of corresponding coefficients for males and females in the tenth generation (tables 4 and 5) shows that variability in body weight was much the same in the two sexes, although the males tended to be slightly more variable in early life.

Although gray females in both the first and the tenth generations were more variable in body weight than albino females, the trend of variability with age in females of the later generation was more like that in albino females, since it reached its maximum at an early age and then declined (table 5).

There were two sources of error in the data for body weight in gray females that probably increased the size of the coefficients of variation for age periods during adult life. Data for a number of females carrying fetuses of sufficient size to add many grams to the mother's weight were doubtless included, since in gray rats pregnancy is well advanced before it is evident from casual observation: weights were never taken, however, if females were known to be carrying young. The data given include the body weights of females that were suckling litters. Lactation is a severe tax on the reserves of a female, especially if the litter is large, and the mother may lose considerable weight at this time. Such errors in the data cannot well be avoided if one is recording, at stated times, the body weights of breeding females; but, since they balance each other to some extent, they do not materially affect the average body weight at a given age for a large series of animals. They do, however, decidedly

influence the size of coefficients of variation for body weight that are calculated from individual, not from average, body weights.

It was not possible to determine the variability in body weight at birth in the gray rats used in this study. Coefficients of variation were calculated from the birth weights of 226 males and 212 females in seventy-one litters cast by gray females of the eighth to the tenth generation, inclusive, in order to obtain some idea of body-weight variability in newborn gray rats. The coefficient for the males was 12.83 $\pm$ 0.40; that for the females was 12.62 $\pm$ 0.42. Variability in body weight at birth was, therefore, about the same in the two sexes. Birth weights in the males were slightly more variable than those in the females, ranging from 3.5 to 7.7 grams in the former and from 3.6 to 7.2 grams in the latter.

Data given above indicate that both in the gray and in the albino race of Norways males tend to be more variable in body weight than females during adolescence and early maturity. A slight, but constant, difference in body-weight variability of the sexes has been noted in other series of Albinos (Jackson, '13; King, '18, '19), and also in mice (Robertson, '16; Saller, '27). These observations are in accord with the theory, held by Darwin (1871) and by Brooks (1883), that throughout the organic world males, in general, are more variable than females.

In man, according to Boas (1897) and Porter ('05), variability in body weight is correlated with the growth rate. A like correlation was found in the stock Albinos used in this study, since variability was greatest at an age period at which growth was very rapid, and then gradually declined as the growth rate decreased during adult life. A rapid growth rate does not necessarily indicate a very high variability in body weight, however, as in both sexes of albino rats the body-weight variability was less than that in gray rats in which the growth rate was relatively slow during early life.

This same correlation is shown, also, by the data for the gray rats. In males of the first generation variability in body weight was highest at the time that the growth rate was most accelerated (60 to 151 days), and it showed no abrupt drop during early adult life when the rate of growth was decreasing but slowly. In these rats variability in body weight closely followed changes in the growth rate throughout life (table 4). In the females of this generation there was only a slight increase in body-weight variability at the sixty-day period when the rats were growing rapidly, and variability increased during adult life when the growth rate was decreasing. In both sexes of gray rats of the tenth generation the correlation between growth rate and body-weight variability was as marked as in the Albinos.

Darwin (1875) was of the opinion that all domesticated animals, with rare exceptions, vary far more than animals in a wild state. He states:

> Variability of every kind is probably directly or indirectly caused by changed conditions of life, or put another way, if it were possible to expose all the individuals of a species during many generations to absolutely uniform conditions of life there would be no variability.

Had the science of genetics been developed during Darwin's time, he would doubtless have modified many of his views, including that of the cause of variability.

In gray rats that have lived for a number of generations in captivity under fairly uniform environmental conditions variability in body weight has tended to decrease. Such a result was to be expected if, as seems probable, some part of this variability was due to the action of factors inherent in the individuals themselves.

Our colony of gray rats is, in a sense, an inbred colony, since no new stock has ever been added and all of the rats have descended from six wild females. These rats have not been very closely inbred, however, since it has always been the rule to select litters for weighing and for breeding stock that were the offspring of parents not closely related. Such a system of breeding would, no doubt, gradually lessen the

variability in body weight due to the action of genetic factors, as the individuals would tend to become more homozygous as the generations advanced. Close inbreeding does not reduce variability in body weight very rapidly, as is shown by the fact that albino rats, inbred brother and sister from the same litter for twenty-five consecutive generations, showed at the end of this period a variability in body weight at different age periods that was but little less than that in outbred albino controls. In these inbred Albinos, however, changed conditions of nutrition undoubtedly acted to increase the variability in body weight in individuals of the later generations (King, '18, '19).

In a given litter of rats the birth weights of members of the same sex often show considerable diversity, and they may differ by more than 100 per cent. Individuals in the litter tend to maintain the order relation in their weights at birth throughout life, as Dunn ('08) determined for albino rats and as records for a large number of litters in different series of rats in our colony show. There are, of course, exceptions to this rule, but they seem to be due, in most cases, to a change in the physical condition of the individuals that is caused by disease or by an excessive accumulation of fat. When rats are very small at birth, their growth seems to be inhibited from the beginning of postnatal life by causes that are constitutional. This is shown in the case of the so-called 'runts' that are found frequently in litters of rats. In these individuals growth continues at a slow rate for some time, but no matter how much care is given, they never exhibit normal vigor, nor do they ever attain the body size normal for a given age (King, '16).

Female rats of the same age, living under similar conditions of environment and of nutrition and breeding at the same season of the year, may cast litters of like size in which the individuals of the same sex show marked differences in their birth weights. On comparing the later growth of such individuals, it has been found that the weight differences at birth are indicative of unlike growth capacities, and that the

rats with the heavier weights at birth tend to maintain their supremacy at all age periods. While variations in the length of the gestation period may account for the unlike birth weights of the members of these litters, they cannot explain the differences in the growth rate and in the ultimate body weights attained which must be due to constitutional causes.

There is, moreover, a constant, though small, sex difference in the birth weights of rats, males tending to be heavier and slightly more variable than females. This sex difference persists throughout life and becomes greater as the individuals grow older, even when environmental and nutritive conditions are the same for all animals. Seemingly, body-weight variability at birth and that found at all age periods during postnatal life in individuals of the same sex, as well as in those of different sex, are indicative of differences in growth capacity which depend, in large measure, upon constitutional causes. Saller's ('27) statement regarding the cause of variability in the body weights of the mouse is doubtless applicable to many, if not to all, animals:

> In der Anlage der Tiere, also genotypisch begründete Unterschiede im Gewichtsverlauf und damit auch für die von ihr erreichte Endgrösse bestehen.

There are, however, many factors that are not genetic that unquestionably influence body weight in the rat and so tend to increase its variability. The age and physical condition of the mother, the size of the litter, and the length of the gestation period, all affect the fetal young and so contribute to the variability found in the birth weights of the newborn (King, '15). In some cases, also, all of the ova liberated at a given period of ovulation are not fertilized at the same time, as is shown most convincingly when superfecundation occurs (King, '13). The advantage thus given to some of the embryos likewise tends to cause variations in the weights at birth.

During postnatal life the growth rate and also the variability in body weight at different age periods are influenced considerably by temperature, nutrition, housing conditions,

amount of exercise, etc. Unfortunately, no way has been found, as yet, to determine how much of the variability shown is due to genetic factors and how much of it depends upon environmental, nutritive, and physiological causes.

With environmental and nutritive conditions as uniform and as favorable as it was possible to make them, with temperature changes in the colony relatively small, and the housing conditions such that the rats could obtain considerable exercise, it would seem that factors such as these could have had but little effect on the growth rate and on the variability in body weight of the captive gray rats. Physiological factors acting during prenatal life could not, of course, be controlled, and they undoubtedly were responsible for much of the variability found in the birth weights. The variability shown at all age periods during postnatal life, however, was probably due, in part at least, to differences in the genetic constitution of the individuals.

THE REPRODUCTIVE PERIOD

Little is known regarding the reproductive life of gray Norways living in their natural habitat. According to Miller ('11), offspring of wild parents, born and reared in captivity, attain sexual maturity at least by the end of the fourth month. Eaton and Stirrett ('23), in agreement with Lantz ('10), maintain that wild gray rats mature at about three months of age and that breeding continues until the animals are about twenty months old. These investigators, however, give no data in support of their assertions. The youngest of the wild rats used as foundation stock for the captive Grays in our colony must have been at least six months old before they began to breed. Possibly the changed conditions incident to captivity retarded the onset of puberty and so delayed reproduction.

In this study the reproductive life of any gray female is considered to have begun with the birth of the first litter for which a record was obtained. Since females of the earlier generations often destroyed their litters at or soon after

birth, there is the possibility that litters were cast which were not recorded. There is the same possible source of error in the data for the last litters cast, as offspring of old females are often born dead and are eaten or lost in the debris of the cage. It has always been part of the routine work in the colony to examine cages containing breeding animals at least once a day in order to make the litter records as complete as possible.

TABLE 6

Showing length of the reproductive period in days in different generations of captive gray rats

GENERATION OF BREEDING FEMALES	EARLIEST AGE AT WHICH A LITTER WAS CAST	LATEST AGE AT WHICH A LITTER WAS CAST	TOTAL LENGTH REPRODUCTIVE PERIOD	AVERAGE AGE WHEN FIRST LITTER WAS CAST	AVERAGE AGE WHEN LAST LITTER WAS CAST	AVERAGE LENGTH REPRODUCTIVE PERIOD
1	144	702	558	266	470	204
2	141	690	549	244	434	190
3	95	586	491	247	431	184
4	111	578	467	210	444	234
5	117	582	465	230	452	222
6	93	581	488	186	406	220
7	97	703	606	242	494	252
8	120	596	476	209	495	286
9	96	646	550	200	450	250
Average	112.6	629.3	516.6	226.0	452.9	226.9

A summary of the data for the reproductive period in females of the first nine generations of captive Grays is given in table 6. Reproduction in females of the tenth generation will be discussed in a future paper.

Only one of the thirty-seven fertile females in the first generation born in the colony cast a litter before she was five months of age; many of them did not bear young until they were over a year old. The average age at which these females began breeding was 266 days, or approximately nine months. As the generations advanced, breeding began at an earlier age, and in the later generations some of the females cast litters when they were less than four months old.

The average ages at which the first and the last litters were cast by females of the different generations are shown by graphs in chart 6. The graphs were constructed from data given in table 6.

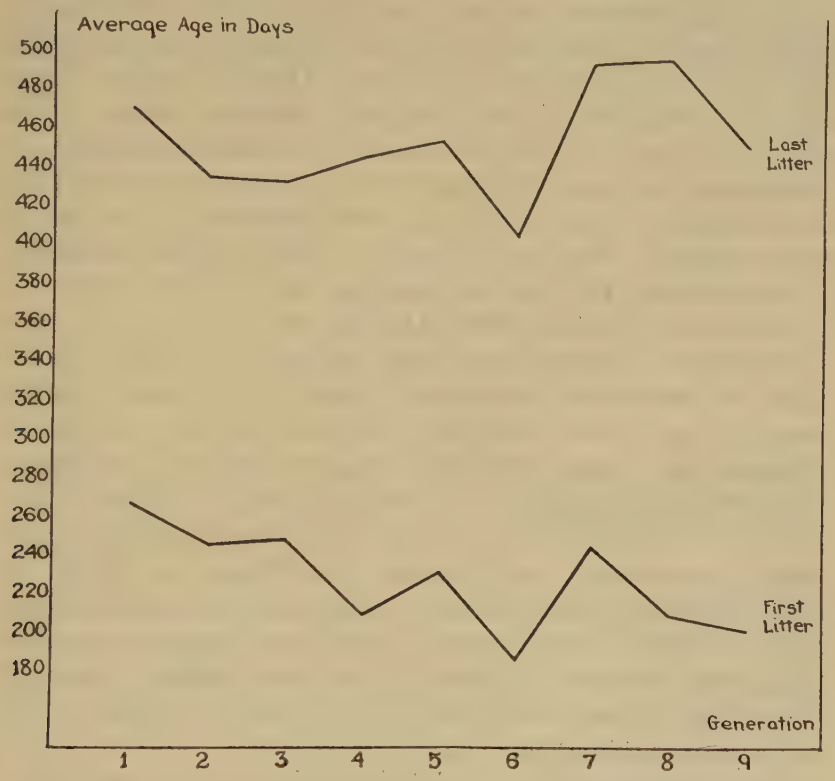

Chart 6 Graphs for captive gray females, showing the average age when the first and when the last litter was cast.

As indicated by the course of the lower graph in chart 6, the average age at which females cast their first litters tended to decrease from the first to the sixth generation. The late beginning of reproduction in females of the seventh generation was due, probably, to the fact that these females did not receive as adequate care as did those of the earlier and of the later generations. With the improvement in general conditions, the females again began breeding at a relatively early

age, as the downward trend of the graph indicates. In females of the ninth generation, reproduction began, on the average, two months earlier than in females of the first generation. The onset of reproductive life in females of the last generation, however, was still considerably later than that in albino females, which cast their first litters, as a rule, when they are about three months of age. It would appear, from the data obtained, either that gray females became more adapted to their changed environment as the generations advanced and so began breeding at or near the time normal for the beginning of reproduction in wild Norways, or, as seems more probable, that life in captivity hastened the beginning of reproductive life, as has been the case in many domesticated animals (Buffon, 1832; Darwin, 1875).

There were marked individual differences in the time at which the menopause appeared in these rats. Many females ceased to bear young after they had cast two or three litters, although they were seemingly in good physical condition; other females continued to breed until they were nearly two years old. While the total length of the reproductive period varied considerably in different generations, as shown in table 6, the variations showed no definite trend as the generations advanced. The average age of the females when the menopause appeared is shown by the upper graph in chart 6. The course of this graph, in general, is much like that of the lower graph on the same chart.

There are, apparently, no definite limits to the reproductive period in female rats. Its length depends, as do growth and many other life processes, on the interaction of a number of different factors. Not only environment, but also the physical condition of the animals, influences reproduction. One factor of paramount importance is the lung infection which frequently attacks rats of both sexes after they are a year old. This disease is not immediately fatal, but it soon puts an end to breeding and so materially shortens the reproductive life of its victims.

The changes in the average length of the reproductive period as the generations advanced are indicated by the graph in chart 7, constructed from data in table 6.

A change in the average length of the reproductive period at the end of three successive generations is indicated by the course of the graph in chart 7. The rise in the graph at the fourth generation denotes an increase of thirty days over the length of this period in females of the first generation.

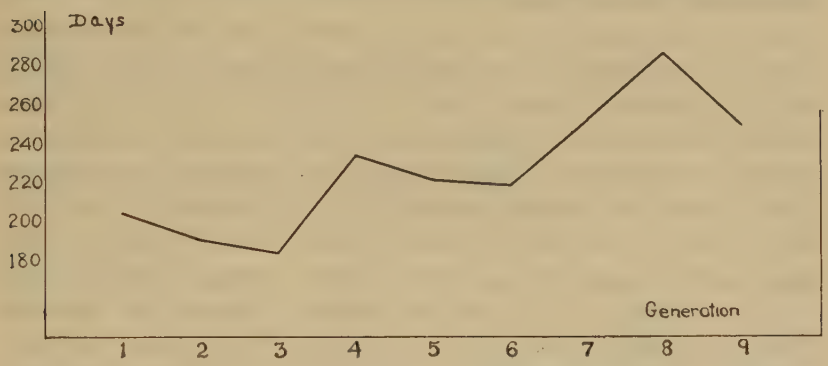

Chart 7 Showing the length of the reproductive period in different generations of captive gray females.

Another upward turn in the graph at the seventh generation is indicative of a further gain of thirty-two days. The highest point in the graph, at the eighth generation, marks a reproductive period extending over 286 days. The average reproductive life of females in the last three generations was seventy days longer than that in females of the first generation.

From the data presented in this section, it appears that the duration of reproductive life in gray females changed considerably in the course of nine generations. Breeding began at an earlier age as the generations advanced, therefore the average length of the reproductive period was increased in later generations by about two months.

FERTILITY

It has long been known from the writings of Buffon (1760), Geoffroy Saint-Hilaire (1862), and Darwin (1875) that domesticated animals, as a rule, are more prolific than their wild representatives. To what extent fertility has been increased has not, however, been determined with certainty for any species, since data for wild forms are meager and, from a statistical standpoint, cannot be considered as adequate for purposes of comparison.

In the rat fertility may be measured both by the number of litters cast and by the number of offspring produced by breeding females during their reproductive life. There are but few statements in literature regarding litter production in wild gray Norways. Zuschlag ('03) assumes eight litters a year to be the average produced by a single pair of rats. Lantz ('10) states that Norway rats probably breed from three to five times a year, while Miller ('11) estimates that five or six litters may be reared yearly. Eaton and Stirrett ('28), from an investigation of the rat population in New Bedford, Massachusetts, are of the opinion that females produce from three to four litters a year. The limitation of numbers in nature is accomplished by a restriction of the reproductive rate, probably through the influence of nutrition, as well as through the action of natural enemies. That inadequate nutrition has a marked effect on reproduction in the rat has been shown by the investigations of Osborne and Mendel ('17), Evans and Bishop ('22 a), and Slonaker and Card ('23). It is to the copious food supply, without the labor of seeking it, that Darwin (1875) attributes the greater fertility of animals under domestication.

The average size of the litters in wild rats has been variously estimated by different investigators. Zuschlag's theoretical table gives eight as the litter average. According to Crampe (1884), Lantz ('10), and Miller ('11), litters contain an average of ten young. Lloyd's ('09) observations on pregnant rats in India showed that litter size ranged from one to twelve, with the average about six. Instances of twenty-

two to twenty-four young in a single nest have been recorded, but there is no evidence that these young were the progeny of a single female. The average number of fetuses in a large number of gravid females captured in India was 8.1 (Lantz, '10). As many as sixteen fetuses were found in wild Norway females by Zuschlag, by Lantz, and by Donaldson ('24). Since many fetuses are absorbed in situ, as Huber ('15) and Long and Evans ('22) have shown, the number of fetuses found in gravid females is not indicative of the number of young that

TABLE 7

Showing litter production and average size of litters in different generations of captive gray rats

GENERATION OF BREEDING FEMALES	NUMBER OF BREEDING FEMALES	TOTAL NUMBER LITTERS	AVERAGE NUMBER LITTERS PER FEMALE	TOTAL NUMBER YOUNG	AVERAGE NUMBER YOUNG PER LITTER
Wild grays	6	21	3.50	139	6.62
1	37	143	3.86	857	5.99
2	42	114	2.71	691	6.06
3	44	142	3.23	849	5.98
4	50	191	3.82	1202	6.29
5	46	176	3.83	1084	6.16
6	48	192	4.00	1167	6.08
7	48	169	3.52	1063	6.27
8	48	210	4.37	1293	6.16
9	48	181	3.77	1160	6.40
	417	1539	3.69	9505	6.17

will be born. All of these observations were made on females of unknown ages, and they give, therefore, no evidence regarding litter production or the average litter size during the reproductive life of the females.

Data showing fertility of females in the different generations of captive Grays are given in table 7.

The breeding record for wild gray females, as given in table 7, may be very incomplete, since some of the females were obviously several months old when they were brought into the colony, and they may have cast young previously to this time. The data for these rats, however, are quite in ac-

cord with those for gray females of the later generations, and they doubtless cover the greater part, if not all, of the offspring produced.

For the entire series of 417 breeding females in the first nine generations of captive Grays, the average number of litters per female was 3.69, and the deviation from this mean in any generation was relatively slight. The largest number of litters cast by any of the females was ten; the smallest number was one. Conditions of captivity, although affording abundant food and protection from enemies, did not increase the average number of litters cast as the generations advanced.

It may be assumed, perhaps, that breeding was restricted in these gray females by the fact that the animals were not completely adapted to conditions of captivity, and therefore did not reproduce at the rate normal for the race in its natural habitat. Since no adequate data for wild Grays are available for comparison, definite conclusions regarding this point cannot be drawn. The average number of litters cast by captive gray females was considerably less than that in a large series of stock Albinos, reared under similar environmental conditions, where the females produced an average of 5.5 litters (King, '24).

The estimation of eight to ten young as the litter average in wild gray rats is undoubtedly much too high, if applied to the entire litter output of any single female or of any group of females. In both the gray and the albino race of Norway rats litter size changes with the age of the female, and litters cast at the beginning and at the end of the reproductive period are much smaller than those cast during the height of breeding activity.

For the entire series of 1539 litters in the first ten generations of captive Grays the average size of the litter was 6.17, and the deviation from this mean in any generation was not significant (table 7). This average accords with the finding of 6.3 young per litter in stock Albinos (King, '24). The range in litter size was from one to fourteen, the largest lit-

ters, as a rule, being cast by females that were from ten to twelve months old.

The trend in litter size as the generations of gray rats advanced is shown graphically in chart 8. The graph was constructed from the data for average litter size given in table 7.

The highest point of the graph in chart 8 is at its beginning, since the litters cast by wild females were, on the average, somewhat larger than those cast by females in the later generations, probably because wild females did not begin breeding until they were relatively old. At the second generation the

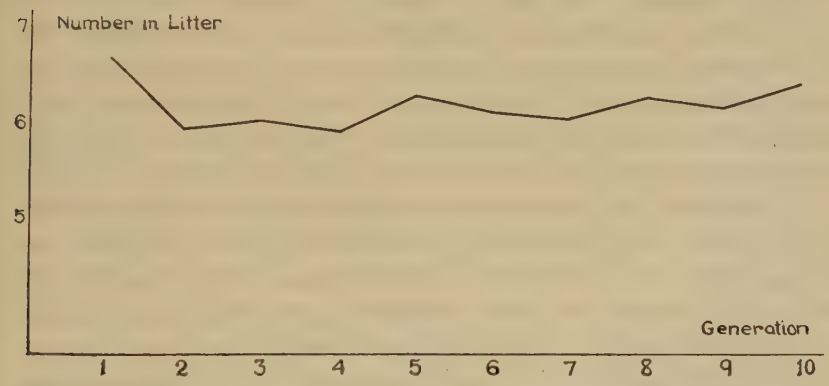

Chart 8 Showing the average size of the litters in different generations of captive Grays.

graph drops to the level that it maintains, with slight fluctuations, until its end.

From the data given above, it is evident that litter production and average litter size in gray rats had not changed appreciably at the end of ten generations of life in captivity. The average number of litters cast by gray females was smaller than the norm for stock albino females, but litter size, on the average, was about the same in the two races.

STERILITY

In commenting on the fact that many wild animals, when brought into captivity, are completely barren or produce but few offspring, Darwin (1875) states that the reproductive

organs of these animals are not diseased, and that the diseases from which they suffer are not those that affect fertility. He shows, furthermore, that this infertility cannot be attributed to failure of the sexual instinct, to a change in climate or in food, nor can it be due to lack of exercise. His conclusion is: "we can in no case precisely say what is the cause of the diminished fertility of an animal when first captured. We can only infer that it is caused by a change of some kind in the natural conditions of life."

The tendency to sterility, which Darwin showed is common to many species of animals when first taken from a state of nature, was exhibited in a marked degree in the case of the wild Norway rats used as foundation stock for our colony of captive Grays. Only six of twenty wild females bore young during the period of their captivity, as far as known. The reproductive power of the females that bred seemed little, if at all, affected by the changed conditions of environment and of nutrition, since the average number of litters they cast and the average size of their litters compared favorably with similar averages for litter production and litter size in later generations (table 7). The fourteen sterile females lived for some months in the colony and appeared to be in good physical condition during this time. Autopsies made on a number of these animals showed no abnormalities or diseases of the reproductive organs, although some of them were found to be suffering from the lung infection which frequently attacks rats of any race after they are a year old. That the changed conditions to which these rats were subjected in some way adversely affected their fertility there can be little doubt.

A factor which may have prevented breeding in many of the wild rats, and one that may be responsible to a greater or a less extent for infertility in other animals when first brought into captivity, was the extreme fear of man which wild rats show in a marked degree. Intense fear produces a nervous tension which undoubtedly influences the activity of many organs of the body, particularly those concerned with secretion. It seems not improbable, therefore, that this nerv-

ous tension, when prolonged, indirectly affects the reproductive organs which are particularly responsive to disturbances of the nervous system. After a time most wild animals become accustomed to life in captivity and they then lose their fear of man to a great extent. With the lessening of nervous tension body organs function more normally. Breeding then takes place at a normal rate, or it is increased in many cases, presumably by the abundant and adequate food that the animals receive and also by the protection from enemies which enables the individuals to live out the normal span of reproductive life.

TABLE 8

Showing sterility in different generations of captive gray rats

GENERATION OF BREEDING FEMALES	TOTAL NUMBER FEMALES REARED	NUMBER FEMALES BREEDING	NUMBER FEMALES STERILE	PER CENT STERILITY
Wild grays	20	6	14	70.00
1	59	37	22	37.29
2	60	42	18	30.00
3	54	44	10	18.52
4	56	50	6	10.71
5	52	46	6	11.54
6	54	48	6	11.11
7	53	48	5	9.43
8	51	48	3	5.88
9	59	48	11	18.64
	518	417	101	19.49

Data given in table 8 show the total number of females reared, the number that did not breed, and the percentage of sterility in each of the nine generations of captive Grays.

As shown in table 8, the sterility in females of the first generation born in captivity was 37.29 per cent, only about half of that (70.00 per cent) in wild females. This percentage is too high, if one takes into consideration the fact that five of the females of the first generation escaped from the colony and were not recaptured. These five females ranged in age from seven to thirteen months at the time of their escape.

None of them had cast young up to this time, but they might have done so later, since many females in the early generations did not breed until they were a year or more old. If these females are omitted from the data in table 8, the sterility for the females of the first generation falls to 31.48 per cent.

The marked decrease in sterility in the offspring of wild females is very significant, since it shows that the majority of them quickly adapted themselves to conditions of life in captivity, which had apparently rendered many of the wild females impotent. Rats of this first generation showed far less fear and less nervous tension than did their wild parents, and all of the females reared their own offspring, which but one of the wild females was able to do.

Sterility decreased steadily as the generations advanced (table 8), and at the fourth generation it had fallen to about 11 per cent, which is the percentage level of sterility in well-cared-for stock Albinos (Slonaker and Card, '23). Sterility reached its lowest point at the eighth generation, where it was but 5.88 per cent. A trend to higher sterility in the stock is not indicated by the increased sterility in the ninth generation. In this generation two of the females died before they were six months old, two others had to be killed because they had ovarian tumors, and three females in one litter developed lung infection at an early age and did not breed although they lived for several months.

The striking decrease in sterility in succeeding generations of these gray rats is shown by the graph in chart 9, constructed from data in table 8.

From the downward trend of the graph in chart 9, it is obvious that the conditions under which these gray rats lived tended to eliminate sterility. In each generation the rats became more adapted to their changed environment, and by the third generation had lost much of their fear of man. They came to the front of the cage when they heard the truck that brought food, and were not frightened when the young were taken out for examination or when the cage was cleaned. The increased fertility in these rats as the generations

advanced can be ascribed, I believe, in part to the lessening of nervous tension, and in part to the fact that the animals were fed on a well-balanced ration containing the elements essential for reproduction.

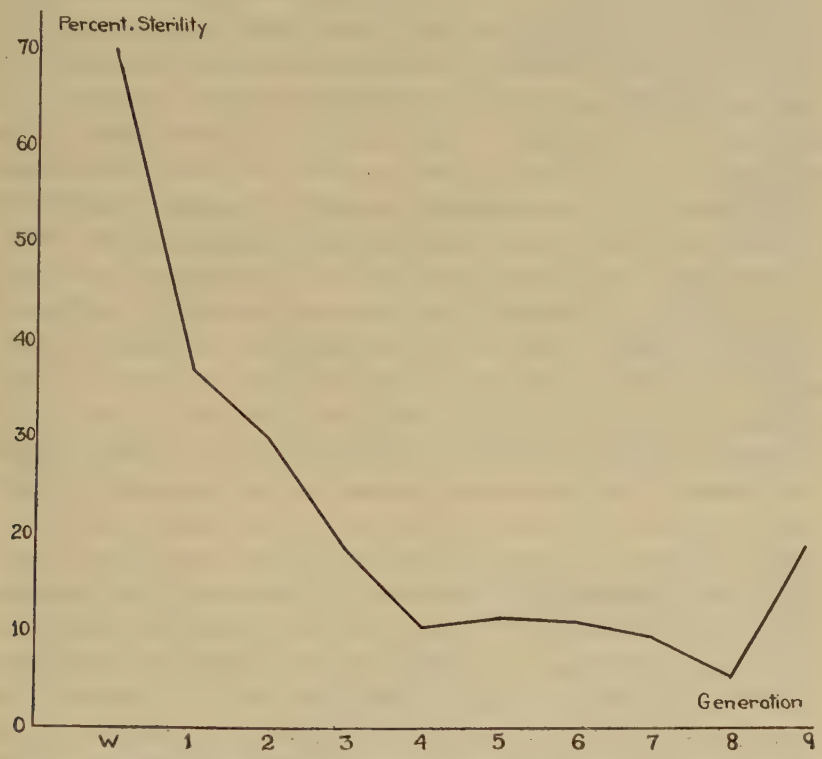

Chart 9 Showing the percentage of sterility in different generations of captive Grays.

Most, if not all, of the sterility found in females of the later generations can be attributed to diseases which affected the reproductive organs or so lowered vitality that the individuals could not breed. Autopsies were not made on rats found dead in the cage, but when it was necessary to kill any individuals because of obvious illness, the fact was noted. Records show that a total of sixteen females belonging in the first nine generations were killed because they had tumors,

chiefly ovarian or mammary. The ear infection, mastoiditis, rendered it necessary to dispose of four other females, while seventeen had to be killed because they had lung disease in an advanced stage.

Some years ago, in collaboration with my colleague, Dr. J. M. Stotsenburg, autopsies were made on a considerable number of albino females, from eight to twenty months old, that had never cast young although they were apparently in good physical condition. These autopsies showed that, in the majority of cases, sterility was due to abnormal or diseased conditions of the reproductive organs. In some females the ovaries were very small and obviously immature; in other females the ovaries were abnormally large, much lobulated, and frequently contained pus; ovarian tumors or cysts were present in other females. A pathological condition of the uterus was the cause of sterility in many cases. In some females the uterus was much congested, the horns containing yellowish nodules of pus; in others the uterus was very small and anemic. An infectious disease of the oviducts, pyosalpinx, was found in some instances. In its early stages lung infection does not prevent conception, but it seriously interferes with embryonic development. Offspring of infected mothers are usually very small at birth, many of them are born dead, and those that live show low vitality and usually die at an early age. As this disease advances, it leads to complete sterility. It seems probable that infertility in female rats is due chiefly to diseases affecting the reproductive organs or to the devitalizing effects of lung infection. Impotency in males is comparatively rare, and when found is caused mainly by diseases, such as lung infection, that lower the vitality of the individuals.

As previously stated, if gray females were in good physical condition they were kept until it was evident that breeding was at an end. Autopsies made on old females that had not cast young for several months frequently showed that a state of pregnancy existed, but that the embryos were in the process of being resorbed. It is evident, from this finding, that ovula-

tion takes place when females are relatively old, and that the ova liberated are capable of being fertilized, but that senescence has produced changes in the uterus that render it impossible for the embryos to undergo normal development. These changes evidently occur at various ages in different individuals, which accounts for the curtailing or the lengthening of the reproductive period, but what they are and how they interfere with embryonic development remains to be determined.

Data given in this section show that sterility in captive gray females had decreased from 70.00 per cent to less than 10 per cent at the end of nine generations. The cause of this decrease can probably be attributed largely to a lessening of nervous tension as the animals became more adapted to changed conditions of environment and to the adequate food that they received. Infertility in females of the later generations was due chiefly to diseases that affected the reproductive organs or to decreased vitality resulting from lung infection.

THE SEX RATIO

The proportion of the sexes at birth in wild Norway rats has not been determined as yet. Data obtained by Miller ('11) showed that there were twenty-three males and twenty-eight females, or a sex ratio of 82.1 males to 100 females, in five litters cast by caged females of unknown ages. Among trapped animals, according to Lantz ('09) and Donaldson ('12), males are in excess, as a rule. This finding does not indicate the relative proportion of the sexes among adult animals generally, however, since the females, if suckling young, probably remain in or close to the nest most of the time and therefore are not as readily trapped as are the males. Sex ratios for gray Norway rats, given in previous publications (King, '24, '27) were based on data obtained from the series of these animals that form the material for the present study.

The sex distribution and the sex ratio in the entire series of 1539 litters comprised in the first ten generations of captive Grays are given in table 9.

As shown in table 9, litters forming the first generation of captive gray rats, which were cast by wild females of unknown ages, contained a total of 139 young, among which the sexes were very evenly distributed, the ratio being 98.57 males to 100 females. In the second generation the ratio dropped to 81.95 males to 100 females. The difference of 16.62 ± 11.94 between the ratios for the first and second gen-

TABLE 9

Showing sex distribution and sex ratio in different generations of captive gray rats

GENERATION	TOTAL NUMBER INDIVIDUALS	MALES	FEMALES	NUMBER MALES TO 100 FEMALES
1	139	69	70	98.57 ± 11.37
2	857	386	471	81.95 ± 3.79
3	691	319	372	85.75 ± 4.41
4	849	418	431	97.18 ± 4.49
5	1202	611	591	103.38 ± 4.02
6	1084	553	531	104.14 ± 4.26
7	1167	570	597	95.47 ± 3.78
8	1063	530	533	99.43 ± 4.11
9	1293	653	640	102.03 ± 3.82
10	1160	571	589	96.94 ± 3.84
	9505	4680	4825	96.99 ± 1.34

erations is considerable, but it cannot be deemed as statistically important because of the size of its probable error. In succeeding generations the sex ratio rose gradually, reaching its maximum, 104.14 males to 100 females, in the sixth generation, after which it declined slightly.

The changes in the sex ratio with the advance of the generations are shown graphically in chart 10.

Aside from its sharp decline at the second generation, the graph in chart 10 shows only minor fluctuations and tends to run at a level which indicates a nearly equal proportion of the sexes.

In the entire series of 9505 individuals the sex ratio was slightly under equality, being 96.99 $\pm$ 1.34. Deviations from this ratio in any generation are not great enough to be considered as significant.

If it is assumed that the sex ratio found in the young of the first generation is about that normal for the race in its wild state, it follows that changed conditions of life in captivity must have influenced the proportion of the sexes in the two succeeding generations, since the sex ratios in these generations were relatively low. Males are seemingly less viable

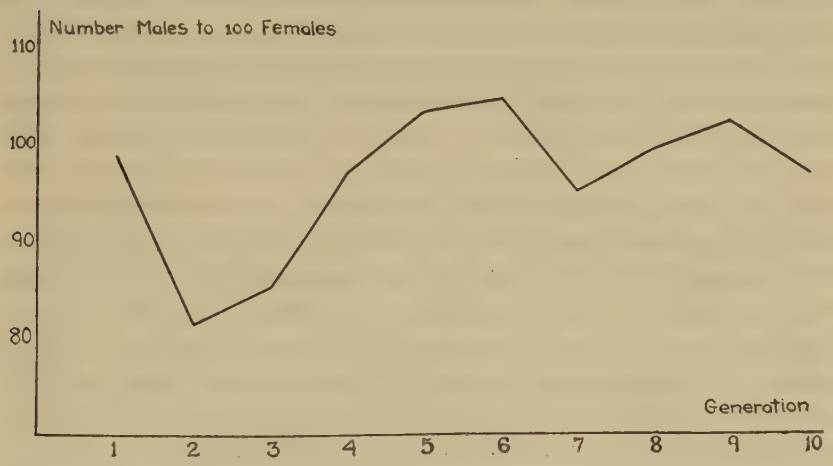

Chart 10 Showing the sex ratio in different generations of captive gray rats.

than females at all stages of development, and their mortality at birth and during early postnatal life is greater than that among females (King, '15). The most obvious explanation, therefore, for the drop in the sex ratio at the second generation is that change in environment rendered fetal mortality selective, and that it bore more heavily on male than on female embryos.

There is, however, another factor which may have affected the sex ratio in the early generations of these rats. It has been shown in a previous publication (King, '24), and the finding confirmed by a large series of data obtained more

recently, that in the albino race the age of the mother has a pronounced influence on the sex ratio in her young. In general, when the data for litters cast by a considerable number of females are examined, it is found that males tend to predominate in litters cast by young mothers and by those that are from ten to fourteen months old, and that female young are in excess, as a rule, in litters produced when the mothers are at the height of their reproductive activity (from six to nine months of age) and when they are approaching the menopause. In what manner the age of the mother can affect the sex proportions in the young is unknown. It may be that changes occur in the uterus at different age periods that tend to favor the development of embryos of one sex rather than those of the other, or there may be age changes in the amount or in the nature of various hormones that at one time render the ova more readily fertilized by spermatozoa that are male-producing and at another time favor fertilization by spermatozoa that are female-producing. Whatever these changes are, they tend to balance each other in the long run. When a female breeds at a normal rate throughout reproductive life, the sex ratio in the entire number of offspring is usually near equality, unless disturbing factors, such as hybridization, produce alterations in the ratio at all periods during the reproductive life of the mother.

As shown in a previous section of this paper, gray females of the early generations began breeding at an average age of about eight months (table 6). At this age albino females are at the height of their reproductive power and their litters contain, as a rule, an increased number of female young. Conditions of captivity, seemingly, eliminated in the gray rats the early period of breeding which seems to favor the development of male young. The reproductive life of these females, therefore, comprised two periods when female young tend to predominate in the litters cast, and but one period when male young tend to be in excess. Their litters, as shown in table 9, contained a greater proportion of female than of male young. As the generations advanced, an increasing

number of females began breeding at an earlier age. The reproductive life of these females included all four periods in which the sex ratio tends to change with the age of the mother. In the young born to these females the sex ratio was near equality (table 9).

Changed conditions of life in captivity apparently had no direct effect on the sex ratio in the litters cast by gray females. They may be considered to have influenced the ratio indirectly, however, by first shortening and then lengthening the average span of reproductive life in the females. From the evidence given it seems more probable that the variations in the sex ratio found in the different generations of these rats can be ascribed to physiological changes due to the advancing age of the mothers rather than to differential mortality during fetal life.

MORTALITY

The scope of this study did not include a determination of the normal duration of life in captive gray rats, as it seemed more important to ascertain the condition of the central nervous system and of various other organs before they had been seriously affected by senescence or by disease. Most of the rats, therefore, were killed and dissected when they reached the age of twenty months. Data regarding the mortality at various age periods and the chief causes of death are given in the present section.

In the entire series of 9505 young born in the first ten generations, only eighteen individuals, ten males and eight females, were found dead at birth. The mortality at this time, about 0.19 per cent, was therefore very low, and much less than that usually found in stock Albinos, where the stillborn form about 2 per cent of all the offspring cast (King, '21). Since all of the litters could not be examined at the time of birth, there is the possibility that the number of stillborn was greater than the records show and that such individuals were eaten by the mothers.

The mortality among the young during the suckling period was considerable, as the mothers frequently destroyed or neglected their young if the nest was disturbed or the young removed for examination. Mortality among the young at this period was still further increased by the fact that the adults in the cage usually crowded into the nest when frightened, and in consequence some of the young were often crushed or suffocated. As the young rats were able to look out for themselves after their eyes were opened, at the seventeenth day of postnatal life, the mortality among them from this time until the end of the suckling period at thirty days was negligible. Litters finally selected for weighing and as parents of the succeeding generation were those in which there were no deaths until the animals had passed the age of sixty days.

Table 10 shows, for each generation, the number of individuals of each sex living at the age of twelve months, the time when reproductive activity has passed its height and the animals have not yet become seriously affected by lung disease. This table also gives the number of animals still living at twenty months of age, together with the percentages of mortality that had occurred at the two ages specified. A summary of the data by generation groups is also included.

In all generations, as the data in table 10 show, mortality was very low during the first year of life, averaging less than 4 per cent in both sexes. Deaths during this period were due, in the main, to accident or to the savageness of some of the rats that caused them to kill other individuals in the cage.

The mortality increased greatly after the rats were a year old, being due chiefly to lung infection. In the first generation 80 per cent of the individuals of both sexes died before reaching the age of twenty months. The high mortality among the older rats of this generation was probably due, in part, to lack of adjustment to conditions of captivity which rendered them very susceptible to disease.

The change in mortality with the advance of the generations is shown in the lower part of table 10, where the data

for the second to the tenth generations are combined in groups of three generations. In the males of the first group (second to fourth generations) the mortality at twelve months was 5 per cent, which was about twice as great as that in the second group (fifth to seventh generations). The mortality in the third group of males (eighth to tenth generations) was 0.6 per cent higher than that in the second group.

TABLE 10

Showing mortality in different generations of captive gray rats

GENERATION	MALES					FEMALES				
	Number individuals	Number living at 12 months	Number living at 20 months	Per cent mortality at 12 months	Per cent mortality at 20 months	Number individuals	Number living at 12 months	Number living at 20 months	Per cent mortality at 12 months	Per cent mortality at 20 months
1	50	48	10	4.0	80.0	52	49	10	5.8	80.8
2	54	50	37	7.4	31.5	60	55	28	8.3	53.3
3	53	53	23	0.0	56.6	54	52	29	3.7	46.3
4	52	48	29	7.7	44.2	55	55	33	0.0	40.0
5	54	52	38	3.7	29.6	52	48	35	7.7	32.7
6	53	51	40	3.8	24.5	54	52	34	3.7	36.6
7	50	50	35	0.0	30.0	53	53	40	0.0	24.5
8	52	50	34	3.8	34.6	51	49	38	3.9	25.5
9	53	52	41	1.9	22.6	59	57	50	3.2	15.3
10	50	48	34	4.0	32.0	52	51	42	1.9	19.2
	521	502	321	3.6	38.4	542	521	339	3.9	37.4
2– 4	159	151	89	5.0	44.0	169	162	90	4.1	46.7
5– 7	157	153	113	2.6	28.0	159	153	109	3.8	31.4
8–10	155	150	109	3.2	29.7	162	157	130	3.1	19.8

In the females, at twelve months, the change in mortality as the generations advanced was relatively slight, falling from 4.1 to 3.1 per cent from the first to the third group.

The mortality at twenty months for males of the first group was 44 per cent, and it dropped subsequently about 14 per cent. In the females at this age the mortality showed a marked decrease as the generations advanced, being 26.9 per cent less in the third than in the first group. From these findings it is evident that conditions of captivity tended to

lengthen the average span of life in these gray rats, since at both twelve and at twenty months of age the mortality was less in individuals of the later generations than in those of the earlier generations.

Data given in table 10 show that there was practically no difference in the mortality of males and of females in the series as a whole. In both sexes, at twelve months, the mortality was about 4 per cent; at twenty months it was about 38 per cent. The mortality in males and females of the different generations at twenty months is shown by graphs in chart 11 constructed from data in table 10.

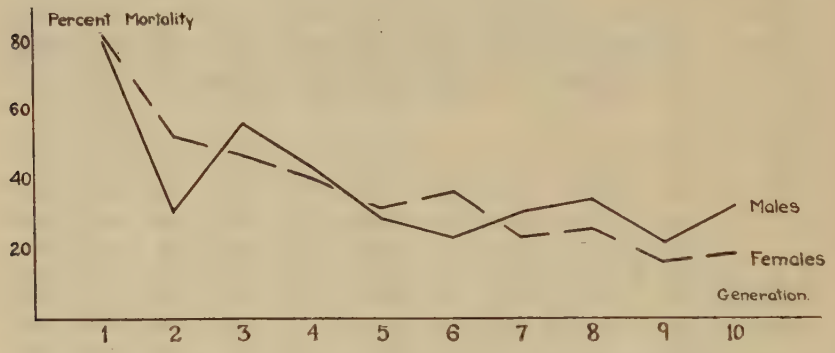

Chart 11 Showing the percentage of mortality at twenty months in males and females of the different generations of captive Grays.

In chart 11 the graph for the females starts slightly higher than that for males, but drops below at the third generation. Subsequently the graphs cross and recross, but after the sixth generation the graph for females runs below that for males until the end of its course. In the later generations, therefore, the mortality was less in females than in males.

Such data as have been obtained indicate that males are less viable than females at birth and during early postnatal life. During adolescence and early maturity there is apparently little difference in the mortality of the two sexes. In later life, however, mortality among males again seems to be greater than that among females. Lung disease, which

is responsible for the majority of deaths among older rats, is seemingly more common in males than in females. On the other hand, cancer and tumors are far more prevalent in females than in males. In the first ten generations of gray rats eighteen females and only one male were killed because of such growths. A third ailment, frequently found in rat colonies, is the middle-ear infection which produces disequilibration and circular movements (Donaldson, '24). Only six cases of this disease were found in the gray rats. The affected individuals were killed as soon as they showed the characteristic symptoms of the disease, as the infection is readily transmitted to other individuals in the cage.

From the findings recorded in this section it appears that in the first ten generations of captive gray rats mortality was very low at birth. It increased during the suckling period, but fell during adolescence and early maturity. After the animals reached the age of one year the death rate rose considerably. As the generations advanced, the mortality at twelve and at twenty months of age decreased in both sexes, but among the older individuals the decrease was more marked in females than in males. The chief causes of death were lung disease and malignant growths.

BEHAVIOR

Since little is known of the general behavior of gray rats living in their natural habitat, it seems worth while to record here the outstanding traits of the wild animals which were brought into the colony to start the strain of captive Grays, and to note the changes in these traits that occurred as the animals became adjusted to their new environment.

When first captured, wild rats were much frightened and so appeared very savage and vicious. They were constantly in a state of high nervous tension due, apparently, to an instinctive fear of man and to the restriction of their activity that captivity imposed. If the cage was approached they began running about, fighting and screaming in obvious terror. When they had been exhausted by such efforts, they

huddled together in a corner of the cage, trembling and clicking their teeth incessantly, or they tried to hide under the bedding material. If the cage door was opened they were apt to jump directly at one's face in a frantic effort to escape. Food put into the cage, no matter how hungry the rats might be, was never touched until the workers in the colony had departed for the day. These rats were very quarrelsome, and some of them were killed and eaten by the more powerful individuals.

The wooden cages that housed these rats had to be protected at all vulnerable points with heavy wire or iron sheeting, as the rats were constantly seeking a way to escape. If they succeeded in loosening the protective covering at any point they would gnaw a large hole through the thick wood of the cage in a few hours. When trapped after such an escape they showed excessive fright for a considerable time. It was several months before these rats became sufficiently accustomed to their new environment to render the work of caring for them and of taking necessary records other than an arduous task.

Miller ('11) has given an account of the mating behavior of gray rats in captivity that is in accord with my own observations. This subject, therefore, need not be considered here. Miller also noted the fact that wild gray females are prone to kill and devour their young at or shortly after birth—a tendency which led to the destruction of a number of litters in our colony also. As already stated, it became necessary to remove the young of the first generation from the cage as soon as they were discovered and to give them to an albino female to rear in order to obtain any litters with which to start the strain.

Since it would have been of distinct advantage in working with these rats of the first generation to have them tame enough to be handled without the use of ether or of forceps, as are albino rats, an attempt was made to tame some of the young that were suckled by albino foster mothers. After the rats were a week old they were taken from the nest each

day and handled gently for a time. They submitted to this handling without much protest until their eyes were opened. After this time they began to bite when held, and they were so frightened and so nervous that the idea of taming them was abandoned. Two young rats of the third generation, a male and female, were taken in charge by an assistant, Miss Ruth Meeser. She succeeded in taming them so that she could handle them at will throughout their lives. They were always very nervous, however, and would not submit to being held by any one who was not a constant worker in the colony.

Females of the first, and those of subsequent generations, reared their own young. It was necessary to wear gloves when the young of the early generations were removed from the nest for examination. If the young were returned to the mother with human odor clinging to them she frequently killed them at once or refused to care for them. In later generations the females did not show much concern if the nest was disturbed and the young handled with bare hands. When they were returned to her, however, she usually carried them to a corner of the cage and built a new nest.

As the generations advanced, the rats gradually lost much of their savageness as well as their fear of man. By the fifth generation they were sufficiently tame to remain quietly in the corner of the cage when the door was opened or the cage cleaned. They came to the front of the cage, as do Albinos, when they heard the sound of the truck that brought food and began eating as soon as food was available. Many of them would take bits of food pushed through the wire of the door, if offered by some one to whom they were accustomed. They were, however, always alarmed at the approach of strangers and promptly went in hiding. Evidently they could distinguish between colony workers and strangers through their keen sense of smell.

It has been the custom, throughout the course of this experiment, to place males of one litter with females of another litter when the young are removed from the mother at the time of weaning. These rats, therefore, grow up together

and they are usually kept together throughout life. Occasionally, for some reason as yet unknown, one of the males in a cage will grow much faster and become considerably larger than its brothers. When this male is powerful enough it dominates the 'home.' The other males are not permitted to mate with the females, as a rule, and they are forced to stay in a corner of the cage away from the nest. Sometimes this dominant male becomes very vicious and develops 'killer' instincts. He then either kills the other males or injures them so severely that they have to be removed from the cage; females are rarely molested in any way. It would appear probable, from this finding, that in a state of nature gray rats are inclined to be polygamous, the most vigorous male driving out or killing the other males and then occupying the burrow with several females. Such a condition exists in many other wild species, as is well known.

Occasionally a female that has become larger than her sisters will exhibit the 'killer' traits shown by males. One such female, weighing over 400 grams, killed two sisters and two males in the cage. She lived peacefully for some months with the remaining male that was considerably larger than herself, and took very good care of her numerous offspring as long as they remained with her.

A most pronounced characteristic of gray rats, and one that time and captivity have not changed to any extent as yet, is their decided aversion to the presence of strange rats. If the inhabitants of the cage are adults, they will at once attack and soon kill the intruder, no matter how large it may be. Even if the new arrival had formerly been housed in the cage, and had been absent but a day or two, it promptly meets with the same fate as that of a total stranger. When a rearrangement of adult rats of mixed sexes is necessary, the only way to insure each of the individuals more than a fair chance of life is to etherize all of the rats, give them a bath in a fairly strong solution of some disinfectant, such as creolin or larkspur, and then place them in a recently sterilized cage containing a quantity of sterilized bedding. The

bath destroys the nest odor of the rats and sterilization removes all other animal odors from the cage and bedding. The rats huddle together under the bedding for some time, dazed by the ether and thoroughly cowed by the treatment they have received. When they have become dry and have ventured from hiding to inspect their new quarters, they are usually friendly and do not fight subsequently. Even this drastic treatment is not always effective, however, and after this procedure it is not uncommon to find on the following day that one or more of the individuals have been killed.

By the end of ten generations in captivity, the behavior of these gray rats had changed considerably in many respects: they had lost much of their viciousness and their fear of man; females rarely killed the young returned to the nest after being handled with bare hands; their nervous tension was distinctly less than that in animals of the earlier generations, although they were obviously frightened when taken from the cage to be weighed or when the cage was approached by those to whom they were not accustomed; they made but little effort to escape when the door of the cage was opened or when the cage was cleaned. Rats of the later generations had not, however, overcome their aversion to the presence of strange individuals, and such intruders were killed as promptly as in the earlier generations.

Although gray rats of the later generations were somewhat more nervous and more easily frightened than are the general run of Albinos, they were far more vigorous and active, and they tended to be larger animals, especially the females. Sterility in these Grays was much less than that commonly found in stock Albinos, and the two races were nearly equal as regards fertility. The life span in the Grays compared very favorably with that in the Albinos, and both races showed about the same susceptibility to lung infection and to other diseases. Because of their size and vigor, these Grays would seem to be superior to Albinos for many laboratory purposes. When captivity has rendered them tame enough to be handled easily, they should be valuable material

for experimental work requiring a pure strain of known ancestry.

SUMMARY

1. The analysis of various life processes in captive gray rats, as given in part I of the present paper, is based on data for the first ten generations of these animals that descended from six wild Norway females brought into The Wistar Institute Animal Colony in the spring of 1919. Data given comprise 1539 litters, containing 9505 individuals, 4680 males and 4825 females.

2. Marked changes in the rate and in the extent of body growth occurred in both sexes of captive Grays as the generations advanced (tables 1 to 3). At the tenth generation growth during early life was much more rapid than that in animals of the first generation, and was approaching the albino type. Growth during adult life changed relatively little, however, as the average body weight of adult males in the tenth generation was only about 4 per cent greater than that of males in the first generation (table 4, chart 4); in adult females the body weight had increased about 8 per cent (table 4, chart 5).

3. The variability in body weight at birth was about the same in both sexes of captive Grays, but the range of variation in the birth weights was slightly greater in males than in females.

4. Males of the tenth generation were significantly less variable in body weight throughout adult life, but not during early life, than were males of the first generation (table 4); females of this generation showed a slight decrease in variability at all age periods (table 5).

5. As the generations advanced, the trend of variability in both sexes of captive Grays was toward that found in stock Albinos. Males of the tenth generation were slightly more variable in body weight during early life than were females, but a reverse relation was found throughout adult life (tables 4 and 5). In both sexes variability in body weight was cor-

related with the growth rate, as is the case in the albino rat and in man.

6. Evidence presented indicates that variability in the body weight of rats during postnatal life is due, in part, to differences in the genetic constitution of the individuals.

7. The average age at which females of the early generations cast their first litters was seven months. With the advance of the generations breeding began at an earlier age; consequently, the average length of the reproductive period was increased by about two months (table 6, chart 7).

8. The average number of litters cast by gray females of the first nine generations was 3.69. Deviations from this mean in any generation were too small to be significant (table 7).

9. The range in litter size was from one to fourteen, with the average 6.1. This average had not changed appreciably at the end of ten generations (table 7, chart 8).

10. Sterility was very high (70 per cent) in the wild females which formed the foundation stock for this series of captive Grays. It decreased steadily, however, as the generations advanced, falling to 5.88 per cent at the eighth generation (table 8, chart 9). Sterility was due chiefly to diseases affecting the reproductive organs or to decreased vitality resulting from lung infection.

11. In the entire series of individuals the sex ratio was 96.99 ± 1.34. Deviations from this ratio in any generation were not statistically important, and they are ascribed, tentatively, to physiological changes due to the advancing age of the mothers rather than to differential mortality during fetal life.

12. Mortality at birth was very low in the first ten generations of captive Grays (0.19 per cent). It increased during the suckling period, fell to a low point during adolescence and early maturity, and showed a marked rise after the animals were a year old.

13. In the series as a whole the mortality was about the same in the two sexes at the age periods noted, being about

4 per cent at the age period of twelve months and 38 per cent at the age period of twenty months (table 10). In the later generations the mortality at twenty months tended to be less in females than in males (chart 11).

14. Mortality decreased steadily as the generations advanced. In males it fell from 80 per cent in the first generation to 32 per cent in the tenth generation; in females the drop was from 80 per cent to 19 per cent. The chief causes of death were lung disease and malignant growths.

15. Marked changes in behavior occurred during the course of ten generations. The rats gradually lost much of their savageness as well as their fear of man, and their nervous tension decreased considerably.

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PART II, SERIES 1. OBSERVATIONS ON THE BODY MEASUREMENTS AND ON THE WEIGHTS OF SOME ORGANS OF CAPTIVE GRAY NORWAY RATS

HENRY H. DONALDSON

MATERIAL AND PROCEDURE

The rats examined were all bred and reared by Doctor King. For this study, series 1, 499 animals have been dissected according to a uniform method by Miss Ruth Meeser. It was planned to use about twenty rats of each sex in each generation, but, as the tables 1 and 2 show, this number has, on the average, been slightly exceeded.

In each case the body length and body weight were determined. The organs weighed were the brain and two of its parts: the olfactory bulbs and the paraflocculi; also the hypophysis, the thyroid, the suprarenals, and the gonads. To interpret these data the values observed for each case were referred to the corresponding values for either the wild gray Norway or the albino rat taken from a series of tables found in The Rat (Donaldson, '24), and the deviations from these standards determined. In the text that follows, these tables are distinguished by the numbers which they bear in the publication just noted and the citations are in italics. Since the effort is here being made to follow not only the way in which the gray Norway rat in captivity departs from the Albino, but also from the wild strain, in respect of the characters studied, it would have been ideal could we have had also a complete series of reference tables for the wild Norway to be used for every comparison. Such reference data, however, are available only for the body length, body weight, and the brain weight. For the other comparisons with the wild strain, it has been necessary to use a series of short tables

compiled by Hatai ('14) giving the weights of the several organs in the wild strain. Taken altogether, these tables indicate the values for the several organs in the wild strain and give a basis for determining the changes shown by the captive Grays.

For conciseness we shall use, in the subsequent text, the term 'captive Grays' for the Norway rats here considered.

In forming the tables and charts that follow, the procedure has been this: The datum for any organ or part of an individual captive Gray is compared with the corresponding datum from the appropriate reference table, the comparison being based either on the observed body weight or body length. Using the datum in the reference table employed as the standard, the percentage deviation, plus or minus, of the observed values for the captive Grays has been determined. The average of all the individual variations in one sex group of a given generation is the datum entered in the condensed tables here presented.[2]

The deviations based on the observed body weight and on the observed body length are both given in the tables. As will be shown later, however, the body weight exhibits a relative increase between G_1 and G_{10}, and for this reason only the deviations based on body length are used for the graphs in the charts. In tables 11 and 12 there are given for each sex of each generation the average ages and the average body lengths, but these data are not repeated in the later tables. In the charts the reference-tables values are represented by the base line and the percentage deviations of the captive Grays are entered in their proper relations to this. The same base line, of course, represents the reference values for both males and females. Any departures from this general arrangement of the tables or charts will be noted as they occur.

BODY LENGTH

The values for the mean body length as given in tables 11 and 12 are entered in chart 12. The fluctuations in the graphs

[2] The individual data are on file at The Wistar Institute, where they may be consulted.

for both sexes have the same trend, except at G_{10}. Between G_3 and G_{10} the maximum difference is about 6 per cent in both sexes.

At G_1 the age of the group is high and at G_2 it is low in both sexes as compared with the subsequent age values. As would be expected, the older group, G_1, has a greater length and the younger, G_2, a lesser length. There is a markedly low length at G_5, and, as later observations will show, this is probably a retardation in growth in response to unfavorable conditions acting on this generation. As the data stand, there is no significant difference at 600 days between the body length at G_3 and at G_{10}, so that there is no evidence that the captive Grays have undergone a progressive change in body length on age during this period.

TABLE 11

General data on the number, age, body length, and body weight of the gray Norway rats used for this study and the deviations of the observed body weight from the body weight as given in the reference table 187 (The Rat, '24) for the observed body length. Charts 12 and 13. Males

GENERATION	NUMBER OF CASES	AVERAGE AGE IN DAYS	BODY LENGTH OBSERVED	BODY WEIGHT OBSERVED	BODY WEIGHT ON BODY LENGTH OBSERVED (TABLE 187)	DEVIATION OF OBSERVED BODY WEIGHT
			Mm.	*Grams*		*Per cent*
Wild	4		188	174	146	+ 16.0
G_1	23	726	241	352	335	+ 4.8
G_2	43	533	234	331	301	+ 9.0
G_3	18	600	240	380	330	+ 13.1
G_4	27	602	249	404	378	+ 6.4
G_5	20	607	235	346	306	+ 11.5
G_6	20	600	247	390	367	+ 5.9
G_7	23	595	246	393	361	+ 8.1
G_8	21	602	237	359	315	+ 12.2
G_9	20	606	236	383	310	+ 19.0
G_{10}	20	606	238	384	320	+ 16.6

TABLE 12

General data on the number, age, body length, and body weight of the gray Norway rats used for this study and the deviations of the observed body weight from the body weight as given in the reference table 187 (The Rat, '24) for the observed body length. Charts 12 and 13. Females

GENERATION	NUMBER OF CASES	AVERAGE AGE IN DAYS	BODY LENGTH OBSERVED	BODY WEIGHT OBSERVED	BODY WEIGHT ON BODY LENGTH OBSERVED (TABLE 187)	DEVIATION OF OBSERVED BODY WEIGHT
			Mm.	*Grams*		*Per cent*
Wild	6		200	184	182	+ 1.1
G_1	18	704	225	275	270	+ 1.8
G_2	26	578	216	256	234	+ 8.6
G_3	26	589	221	269	253	+ 5.9
G_4	24	605	225	265	262	+ 1.1
G_5	18	608	220	276	249	+ 9.8
G_6	18	597	232	326	300	+ 7.9
G_7	21	596	225	287	270	+ 5.9
G_8	22	596	225	304	270	+ 11.1
G_9	20	605	224	308	265	+ 13.9
G_{10}	21	607	221	302	253	+ 16.2

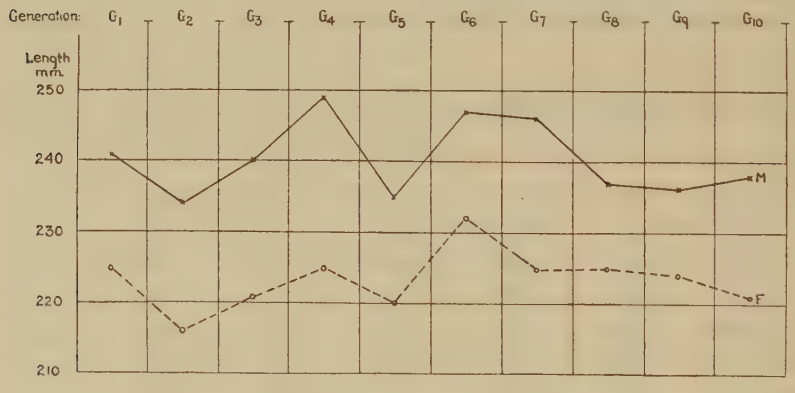

Chart 12 Body length in millimeters. ———, male; – – – –, female.

BODY WEIGHT—ON OBSERVED BODY LENGTH—STANDARD: WILD GRAY NORWAY. *Reference table 187*

When the data in the last columns of tables 11 and 12 are plotted in chart 13, it is seen that the body weight of the captive Grays tends to increase from G_1 to G_{10}. The two sexes behave in a similar manner, though the male is a trifle more

responsive, and at G_{10} the body weight of the captive Grays exceeds that of the wild strain by 17 per cent in the males and by 16 per cent in the females.

If data for the wild gray Norway, taken from *reference table 187,* are compared with data for the Albino, taken from *reference table 144,* using the body lengths that belong to each sex of the captive Grays, it appears that at G_{10} the body weight of the Albino exceeds that of the wild gray Norway by 15 per cent in the male and by 19 per cent in the female.

Thus, the deviation in the body weight of the captive Grays from that of the wild gray Norways is similar to the deviation shown by the Albino. The captive Grays have, therefore, approximated the Albino in this relation. It seems most probable that lack of exercise and the supply of abundant food are the influences which have brought about this modification.

Attention should be called at this point to the sag in the graphs in chart 13, at G_6 and G_7. A corresponding sag occurs in the graphs for most of the other organs, and in the present instance is to be explained as the result of unfavorable conditions acting on these generations (chart 1).

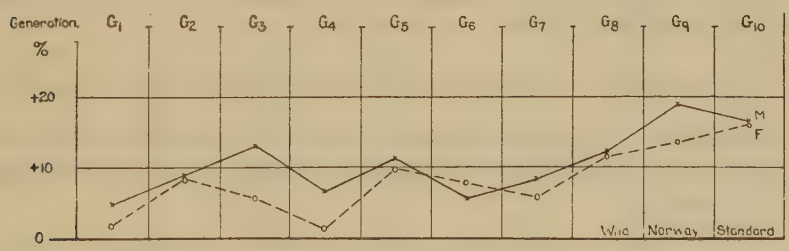

Chart 13 Deviations of observed body weight from standard body weight on observed body length. Standard: Wild Norway, table 187.

ON THE WEIGHTS OF THE SEVERAL ORGANS IN THE CAPTIVE GRAYS

Introduction

There are two ends in view. In the first place, it is the purpose of this study to determine whether the captive Grays have departed from the standards for the wild strain.

For this purpose the observed data are compared with those for the brain, *reference table 187,* or with those for the other organs of the wild Norway in the tables from Hatai ('14).[3] These latter are partial tables only, but can, nevertheless, be used for our present purpose. Such comparisons show how the data for the captive Grays differ from those for the wild strain.

In the second place, we wish to determine how the data for the captive Grays are related to those of the Albinos. To do this the data in the several reference tables for the Albino are used as standards, and the deviations of the observed values similarly determined.

In the tables giving the data on the organ weights there are entered the percentage deviations of the observed from the standard values on both the body weight and body length, as observed. In the charts, however, the graphs for the deviations based on body lengths are alone given.

There are no reference tables for the weights of the olfactory bulbs or of the paraflocculi, and the observations on these parts of the brain have, therefore, been presented from the standpoint of their relation to the observed weight of the entire brain. In the case of the hypophysis, this relation has also been shown.

ON THE WEIGHT OF THE BRAIN AND OF SOME OF ITS PARTS

Standard: Data in *reference table 187* for the wild gray Norway. In the wild gray Norway the brain is heavier than in the Albino.

In tables 13 and 14 the weight of the brain in the successive generations is given for each sex and also the percentage deviations from the standard values. No tables are given for the limiting values in each generation, as shown in charts 15 and 16. In table 15 are given the weights of the olfactory bulbs and of the paraflocculi and the percentage values of the

[3] For convenience, the tables from Hatai ('14) are cited under the numbers which designate them in The Rat (Donaldson, '24).

brain weight represented by them. The data for these parts do not begin until G_3.

In chart 14 the percentage deviations of the brain weight are entered and also the percentage of the weight of the brain represented by the olfactory bulbs and by the paraflocculi. In charts 15 and 16 the limiting values for the deviations in brain weight are shown for each generation. Examination of chart 14 shows that the brain weight in the captive Grays is slightly deficient, but with fluctuations.

These deficiencies tend to follow the same course in the two sexes, but are slightly greater in the males, and in both sexes slightly greater in the last five generations, even when the entries at G_7 and G_8, which mark a sag in the graph, are excluded.

TABLE 13

On the weight of the brain in the captive Grays and on the deviations from the corresponding standards for body weight and body length. Chart 14. Males

GENERATION	NUMBER OF CASES	BRAIN WEIGHT				
		Observed	Standard on body weight observed	Standard on body length observed [1]	Percentage deviation from standard	
					On body weight	On body length
		Grams	*Grams*	*Grams*		
G_1	23	2.190	2.342	2.337	— 6.5	— 6.3
G_2	43	2.138	2.284	2.267	— 6.4	— 5.7
G_3	18	2.237	2.363	2.329	— 5.3	— 4.0
G_4	27	2.317	2.380	2.378	— 2.7	— 2.6
G_5	20	2.198	2.338	2.301	— 6.0	— 4.5
G_6	20	2.255	2.376	2.364	— 5.1	— 4.6
G_7	23	2.182	2.379	2.361	— 8.3	— 7.6
G_8	21	2.150	2.356	2.312	— 8.7	— 7.0
G_9	20	2.196	2.379	2.305	— 7.7	— 4.7
G_{10}	20	2.205	2.374	2.320	— 7.1	— 4.9
					Average:	— 5.2

[1] As the values for the brain weight on observed body length are averages for the values in table 187 for the brain weight on the body length of each individual case, and as this assumes that the graph between the extremes is a straight line, which it is not, these averages differ slightly, by tenths of a per cent, from the values to be expected for the average body lengths that have been used. This deviation is neglected. The same comment applies to the other organ values in the several tables.

TABLE 14

On the weight of the brain in the captive Grays and on the deviations from the corresponding standards for body weight and body length. (Chart 14.) Females

GENERATION	NUMBER OF CASES	BRAIN WEIGHT				
		Observed	Standard on body weight	Standard on body length	Percentage deviation from standard	
					On body weight	On body length
		Grams	*Grams*	*Grams*		
G_1	18	2.105	2.213	2.207	— 5.1	— 4.9
G_2	26	2.124	2.188	2.161	— 2.9	— 1.8
G_3	26	2.094	2.206	2.192	— 5.1	— 4.5
G_4	24	2.146	2.199	2.208	— 2.4	— 2.8
G_5	18	2.093	2.209	2.179	— 5.3	— 4.0
G_6	18	2.172	2.273	2.249	— 4.4	— 3.4
G_7	21	2.072	2.229	2.208	— 7.1	— 6.2
G_8	22	2.069	2.250	2.210	— 8.0	— 6.4
G_9	20	2.105	2.258	2.209	— 6.8	— 4.7
G_{10}	21	2.139	2.248	2.192	— 4.9	— 2.4
					Average:	— 4.1

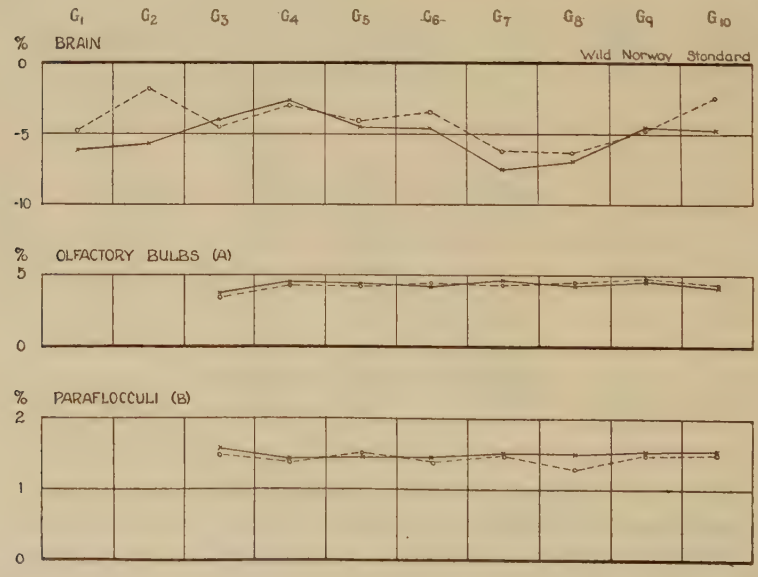

Chart 14 Brain weight—deviations on body length. Standard: Table 187 for wild Norway. ———, male; – – – –, female.

Chart 14 a Percentage weights of olfactory bulbs on brain weight.

Chart 14 b Percentage weights of paraflocculi on brain weight.

The captive Grays show, therefore, a slightly smaller brain weight than the wild strain, but the terminal values at G_{10} are, if anything, a little above those at G_1, so that there is no evidence of a progressive decrease in brain weight in the successive generations. The low values at G_7 and G_8 are considered as due to the unfavorable conditions in the colony which acted on these generations during the early phases of their development. It will not have escaped notice that the weight of the brain in the original ancestors of the captive Grays has not been considered. The data which we have indicate a brain weight about that of the standard in the male and some 3 per cent below the standard in the female, but the number of cases is too small to give anything satisfactory in the way of an average. The data suggest that there was a sudden drop in the brain weight at G_1. What this means, if it occurs, is not at the moment clear.

In view of the paucity of the data for the ancestors, the deviations which may occur between them and the G_1 generation are not considered in the discussion that follows, and any changes which are recorded are referred to G_1 as the starting-point.

The slightness of the reduction in the brain weight of the captive Grays was a surprise, as a much greater reduction had been expected. This expectation was based on the fact that when the data for the wild gray Norway (*reference table 187*) are compared with the data for the Albino (*reference table 144*), using the body lengths found for the captive Grays at G_{10}, the wild gray Norway surpasses the Albino in brain weight by 15 per cent in the males and 14 per cent in the females. It is evident, therefore, that the reduction of the brain weight of the captive Grays at G_{10}, which is 5 per cent or less in both sexes, marks only a slight shift toward the relations characteristic for the Albino. To determine whether the low brain weights characteristic of the Albinos were shown by any individuals among the captive Grays, the graphs in charts 15 and 16 have been prepared. The limiting graphs give the extreme deviations plus and minus for each

sex in each generation. In a general way, these graphs for the extremes follow those for their respective means. They show also that in the case of the males a deficiency of 15 per cent or more occurs five times, while in the case of the females a deficiency of 14 per cent or more occurs three times. Thus, some individuals do deviate as much as do the Albinos on the average, but, as the results show, these extreme deficiencies have not sufficient frequency to produce more than a slight average deficiency in the case of the captive Grays.

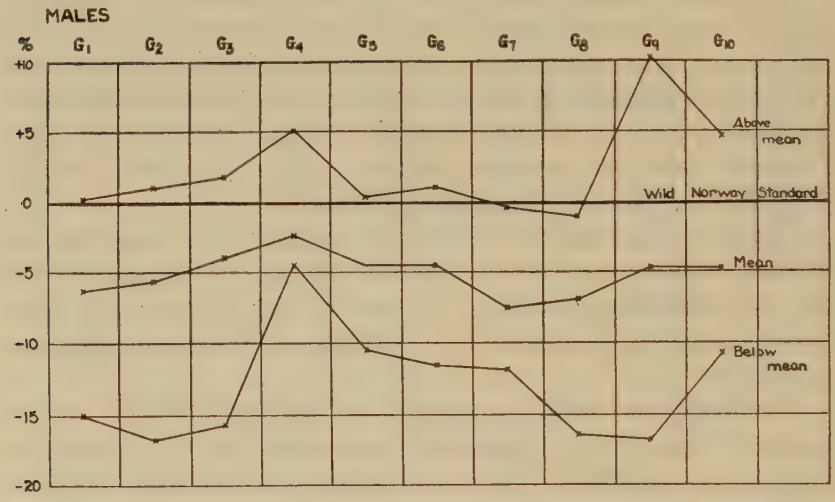

Chart 15 Brain weight: Deviations of limiting values above and below the mean in each generation of captive Grays. Males.

Parts of the brain

The percentages of the brain weight represented by the olfactory bulbs and by the paraflocculi are given in table 15 and chart 14. The relations of these percentages can be briefly stated. In both instances the average of the percentage values for the female runs a trifle below that for the male, but in both sexes and for both parts these values remain nearly constant from G_1 to G_{10}. It follows from this that the fluctuations in the weight of the entire brain are accompanied by corresponding fluctuations in the weights of these parts, thus maintaining constant weight relations.

From this study we conclude, therefore, that captivity for ten generations has reduced the brain weight of the test Grays only by 5 per cent in the males and 3 per cent in the females. The shift is toward the relations characteristic for the Albino, but still leaves some 10 or 11 per cent to go. The data as they stand do not show any marked tendency to a progressive reduction in brain weight. As to the parts of the brain here examined, these vary as does the remainder of the brain, and hence retain a constant percentage weight.

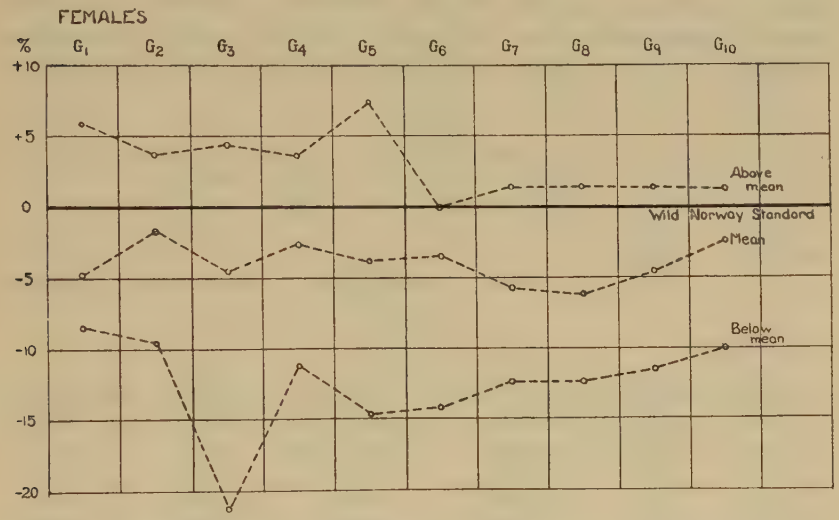

Chart 16 Brain weight: Deviations of limiting values above and below the mean in each generation of captive Grays. Females.

ON THE WEIGHT OF THE HYPOPHYSIS IN THE CAPTIVE GRAYS

Standards: Data in *reference table 148* for the Albino and data in *table 205* for the wild Norway. The hypophysis is lighter in the wild gray Norway than in the Albino. This difference is specially marked in the female hypophysis.

The deviations in the weight of the hypophysis of the captive Grays from albino standards are given in tables 16, 17, and 18 and chart 17. The course of the graphs for the two sexes is similar. When the base line used for reference repre-

sents male albino values, then the values for the male captive Grays, graph 1, run from 10 to 20 per cent minus, while the values for the female captive Grays (3) run about 5 to 10 per cent plus. When, however, the base line in chart is taken as representing the standard values for the female Albino, then the values for the female captive Grays run from 50 to 60 per cent low (graph 2, chart 17).

If the sag which occurs in the graphs at G_6 and G_7 is neglected, the graphs give no evidence for a progressive change in the weight of the hypophysis in the later generations. Nevertheless, at G_9 there is, in both sexes, a conspicuously high value that is noteworthy and may be significant.

TABLE 15

Weights of bulbs and of paraflocculi together with their respective percentage weights on the entire brain. (Chart 14, A and B)

GENERATION	NUMBER OF CASES	BULBS (A)		PARAFLOCCULI (B)	
		Observed	Per cent brain weight	Observed	Per cent brain weight
Males					
		Grams		*Grams*	
G_3	10	.0868	3.88	.0342	1.54
G_4	27	.1064	4.57	.0329	1.40
G_5	20	.0958	4.35	.0322	1.46
G_6	20	.0976	4.34	.0324	1.44
G_7	23	.1045	4.78	.0323	1.48
G_8	21	.0902	4.20	.0320	1.49
G_9	20	.0985	4.50	.0329	1.50
G_{10}	20	.0947	4.24	.0333	1.51
		Average:	4.36	Average:	1.48
Females					
G_3	17	.0745	3.54	.0304	1.46
G_4	24	.0945	4.39	.0298	1.38
G_5	18	.0864	4.21	.0306	1.46
G_6	18	.0947	4.36	.0299	1.38
G_7	21	.0911	4.37	.0305	1.44
G_8	22	.0922	4.49	.0276	1.29
G_9	19	.1002	4.77	.0303	1.44
G_{10}	21	.0914	4.27	.0320	1.50
		Average:	4.29	Average:	1.42

TABLE 16

On the weight of the hypophysis in the captive Grays and the deviations from the corresponding standards for body weight and body length.
(Chart 17 (1).) Males

GENERATION	NUMBER OF CASES	HYPOPHYSIS				
		Observed	On body weight	On body length	Percentage deviations	
					On body weight	On body length
		Grams	*Grams*	*Grams*		
G_1	23	.0112	.0118	.0125	— 5.3	— 10.0
G_2	40	.0096	.0112	.0119	— 14.1	— 18.7
G_3	18	.0105	.0122	.0123	— 13.9	— 14.6
G_4	27	.0112	.0126	.0135	— 11.1	— 17.0
G_5	20	.0099	.0115	.0118	— 13.9	— 16.1
G_6	20	.0104	.0124	.0135	— 16.1	— 23.0
G_7	23	.0103	.0126	.0133	— 18.3	— 22.6
G_8	21	.0099	.0119	.0121	— 16.8	— 18.2
G_9	20	.0107	.0125	.0118	— 14.4	— 9.3
G_{10}	20	.0091	.0122	.0123	— 25.4	— 26.0

TABLE 17

On the weight of the hypophysis in the captive Grays and the deviations from the corresponding standards for body weight and body length.
(Chart 17 (2).) Females

GENERATION	NUMBER OF CASES	HYPOPHYSIS				
		Observed	On body weight	On body length	Percentage deviations	
					On body weight	On body length
		Grams	*Grams*	*Grams*		
G_1	18	.0109	.0195	.0238	— 44.3	— 54.3
G_2	26	.0104	.0184	.0210	— 43.4	— 50.4
G_3	26	.0104	.0191	.0221	— 45.6	— 52.9
G_4	24	.0104	.0190	.0229	— 45.3	— 54.6
G_5	18	.0104	.0196	.0216	— 46.9	— 51.9
G_6	18	.0110	.0236	.0265	— 53.4	— 59.0
G_7	21	.0102	.0205	.0232	— 50.2	— 56.0
G_8	22	.0107	.0219	.0238	— 51.1	— 55.0
G_9	20	.0117	.0213	.0223	— 45.1	— 47.5
G_{10}	21	.0099	.0217	.0221	— 54.4	— 55.2

TABLE 18

Hypophysis weights. Deviations of female values for the captive Grays when referred to male albino standards. (Chart 17 (3).) Females

GENERATION	NUMBER OF CASES	HYPOPHYSIS		
		Observed	On body length	Percentage deviations
		Grams	*Grams*	
G_1	18	.0109	.0102	+ 6.4
G_2	26	.0104	.0090	+ 13.4
G_3	26	.0104	.0097	+ 6.7
G_4	24	.0104	.0102	+ 1.9
G_5	18	.0104	.0095	+ 8.6
G_6	18	.0110	.0112	— 1.7
G_7	21	.0102	.0102	0
G_8	22	.0107	.0102	+ 4.9
G_9	20	.0117	.0101	+ 13.6
G_{10}	21	.0099	.0097	+ 2.0

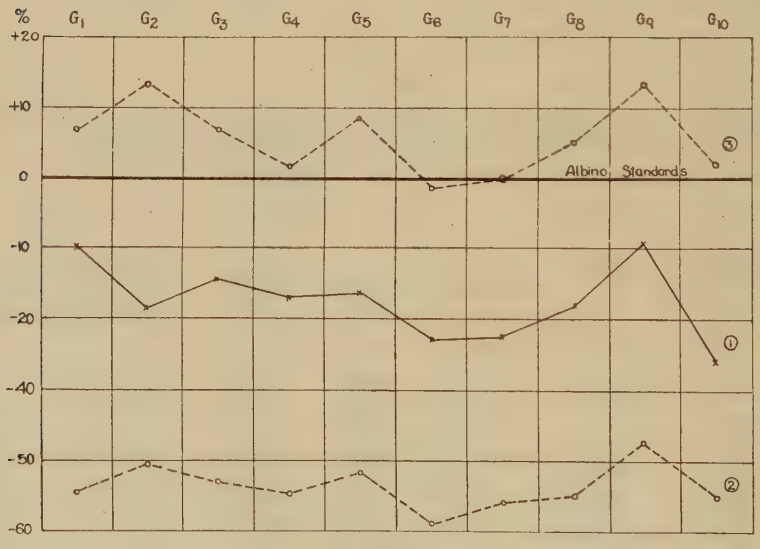

Chart 17 Hypophysis weight: Deviations in captive Grays on body length—Albino standards.

1. For male—when standard Albino values are for the male.
2. For female—when standard Albino values are for the female.
3. For female—when standards are as in 1.

When the percentage weight of the hypophysis on the weight of the brain (data for brain weight in tables 13 and 14 and for the hypophysis weight in tables 16 and 17) is computed, the values obtained are represented by the graphs in chart 18. These graphs show the percentage value of the hypophysis to be higher in the female than in the male, but on the whole the graphs for both sexes are nearly horizontal, indicating a constancy in the percentage values.

To determine whether the weight relations of the hypophysis in the captive Grays have been changed from those shown by the wild strain, the weights for the former have been compared with those for the latter as given in *table 205*. When

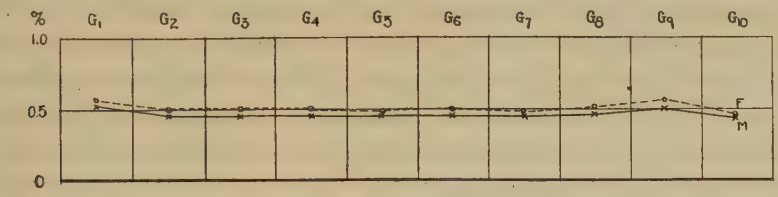

Chart 18 Percentage weight of hypophysis on observed brain weight in captive Grays—both sexes. ———, male; – – – –, female.

the data in that table are revised in terms of body length, it appears that the weight of the hypophysis in the captive Grays runs above that for the wild strain by about 7 per cent in the males and 11 per cent in the females. Thus, the weight of the hypophysis has apparently increased slightly in both sexes of the captive Grays. This is a shift toward the albino values. At the same time the weight relations according to sex remain in the captive Grays similar to those found in the wild strain, and the female hypophysis shows no tendency to the very heavy weight characteristic of the albino females.

ON THE WEIGHT OF THE THYROID IN THE CAPTIVE GRAYS

Standards: *Reference table 148* for the Albino and *table 204* for the wild gray Norway. The thyroid has about the same weight in the wild gray Norway and the Albino.

The percentage deviations in the weight of the thyroid are given in tables 19 and 20. The graphs appear in chart 19.

A word of explanation touching the standard albino record is necessary. The values are from *reference table 148*. This table was made some fifteen years ago, when the Albinos used for it were living under less favorable conditions than those at present obtaining in our colony. One result of the improved treatment has been to diminish the weight of the thyroid in the Albino, so that to-day, at the body length of the captive Grays, the observed values for albino thyroid are 52 per cent below the reference-table values for the males and 53 per cent below for the females.

Inspection of chart 19 shows a progressive diminution in the weight of the thyroid with some fluctuations. In general, the graphs for the two sexes run the same course, but the average percentage deviation for the females is about 1 per cent more than that for the males. This relation, according to sex, calls for a word of comment. On body length the weights for the female thyroids of the standard Albino surpass those for the males by about 7 per cent, while in the wild strain this sex difference does not appear.

Since the basal values for the female Albino are 7 per cent higher than for the male and since the thyroid weight in the captive Grays is only 1 per cent below that of the males when thus charted, it follows that in the captive Grays the thyroid on body length is heavier in the females than in the males. There is thus a shift toward the sex relations found in the Albino.

When compared with the data in *table 204* (reduced to the body-length basis), it appears that the wild gray Norways have thyroid weights close to those for the Albino. From this it follows that the deviation in weight shown by G_1 of the captive Grays probably represents a marked loss in thyroid weight from that for the wild ancestors. It is evident that in the case of the thyroid there is a progressive loss in weight, in both sexes, from G_1 to G_{10}. This has occurred during the period of captivity and might be regarded as an effect of captivity were it not for the behavior of the thyroid in the Albino. In 1912, this albino strain, after many years in

captivity, gave thyroid weights as entered in *reference table 148*. Since then, however, the weight of the thyroid in these domesticated Albinos has shown a marked diminution and to-day (at the body lengths of the captive Grays) is only some 48 per cent (male) and 47 per cent (female) of its original table value. Here, then, is a marked change in the thyroid weight of the Albino occurring after many generations in captivity. This must be due to some special condition, and it seems most probable that some change in the food or its method of preparation has acted as the cause.

It appears, therefore, that the weight of the thyroid undergoes a progressive decrease in the captive Grays, probably in response to some special food conditions. There is a sag in the graphs at G_6 and G_7. The final weight values at G_{10} drop below those for the Albino as given by our current records, while the weight relations according to sex become those for the albino strain (i.e., on body length the female thyroid is heavier).

In this connection we enter some observations by Miss Meeser on structural deficiencies observed in the thyroid and

TABLE 19

Thyroid weights in the captive Grays and deviations from the corresponding standards on body weight and body length. (Chart 19.)

Albino standard. Males

GENERATION	NUMBER OF CASES	THYROID				
		Observed	On body weight	On body length	Percentage deviations	
					On body weight	On body length
		Grams				
G_1	15	.0301	.0501	.0540	— 39.9	— 44.2
G_2	34	.0320	.0477	.0493	— 32.9	— 35.1
G_3	17	.0315	.0533	.0533	— 40.5	— 40.9
G_4	27	.0301	.0560	.0601	— 46.2	— 39.9
G_5	20	.0257	.0495	.0499	— 48.0	— 48.4
G_6	20	.0264	.0544	.0585	— 51.4	— 54.8
G_7	23	.0248	.0548	.0577	— 54.7	— 57.0
G_8	21	.0248	.0516	.0512	— 51.9	— 51.5
G_9	20	.0265	.0536	.0506	— 50.5	— 47.6
G_{10}	20	.0235	.0538	.0519	— 56.3	— 54.7
					Average:	— 47.41

TABLE 20

Thyroid weights in the captive Grays and deviations from the corresponding standards on body weight and body length. (Chart 19.)
Albino standard. Females

GENERATION	NUMBER OF CASES	THYROID				
		Observed	On body weight	On body length	Percentage deviations	
					On body weight	On body length
		Grams				
G_1	7	.0344	.0413	.0467	— 16.7	— 26.3
G_2	22	.0272	.0391	.0414	— 30.4	— 34.3
G_3	26	.0232	.0405	.0443	— 42.7	— 47.6
G_4	24	.0197	.0401	.0467	— 50.8	— 57.8
G_5	18	.0213	.0414	.0437	— 48.5	— 51.2
G_6	18	.0227	.0472	.0480	— 51.9	— 52.7
G_7	21	.0201	.0427	.0467	— 54.9	— 56.9
G_8	22	.0224	.0447	.0467	— 49.9	— 52.0
G_9	20	.0252	.0451	.0461	— 44.1	— 45.3
G_{10}	21	.0171	.0445	.0443	— 61.5	— 61.4
					Average:	— 48.53

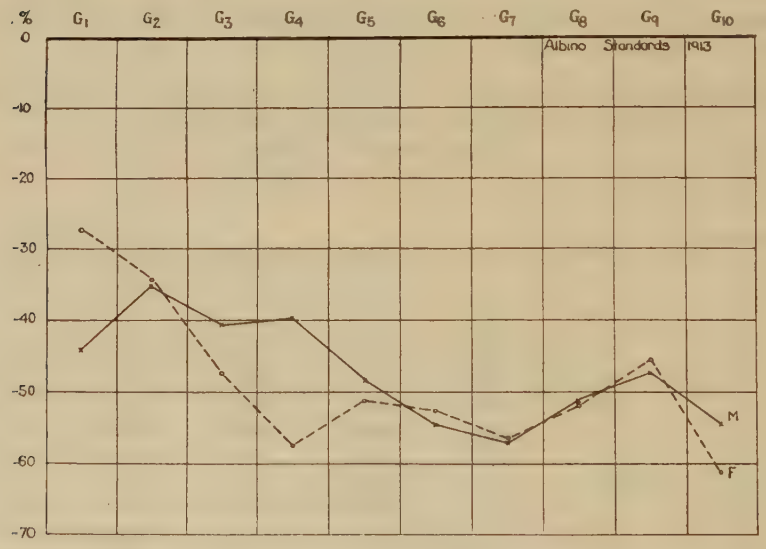

Chart 19 Thyroid weight in captive Grays. Percentage deviation in weight of thyroid on body length—Albino standards—1913. (Tables 19 and 20.)

parathyroid glands. Her dissection records show the relations given in table 21.

It appears that in the generations of captive Grays from G_2 to G_{10} the thyroid was missing on one side in twelve cases and defective in two others. The total number of rats examined was 449. In five cases the corresponding parathyroid was also missing. In thirteen out of fourteen cases the defect was on the right side, and twelve of the fourteen rats were females. Here is a series of defects, in the thyroid and parathyroid, which occur predominately in females and on the right side. The parathyroid is missing in less than half the cases. Four of these five cases of missing parathyroid were in G_{10}.

The skulls which were prepared from this generation showed several instances of defective incisors—a condition probably related to deficiency in parathyroid secretion (Hammett, '22). It may be said, further, that these structural defects seem, so far, to be a peculiarity of this series of captive Grays and have been noted heretofore only in one instance, a hybrid—Norway × Albino—out of hundreds of rats dissected.

TABLE 21

Record of deficiencies in the thyroid and parathyroid glands. Captive Grays—fourteen cases

GENERATION	SEX	THYROID		PARATHYROID	
		Side	Condition	Side	Condition
G_2	F	L	Almost gone	L	Present
G_4	F	R	Missing	R	Present
G_4	F	R	Missing	R	Present
G_5	M	R	Missing	R	Enlarged
G_5	F	R	Missing	R	Present
G_5	F	R	Very tiny	R	Present
G_6	M	R	Missing	R	Missing
G_7	F	R	Missing	R	Present
G_8	F	R	Missing	R	Present
G_9	F	R	Missing	R	Present
G_{10}	F	R	Missing	R	Missing
G_{10}	F	R	Missing	R	Missing
G_{10}	F	R	Missing	R	Missing
G_{10}	F	R	Missing	R	Missing

WEIGHT OF BOTH SUPRARENALS IN THE CAPTIVE GRAYS

Standards: *Reference table 148* for Albino and *table 206* for wild gray Norways. In both the Albino and the wild gray Norway the weight of the suprarenals is much greater in the female than in the male.

In the wild strain the weights in both sexes are greater than in the Albino.

The percentage deviations in the weight of the suprarenals (both glands) are given for the captive gray males in table 22 and for the females in table 23. The graphs for the deviations on body length are entered in chart 20. Inspection of

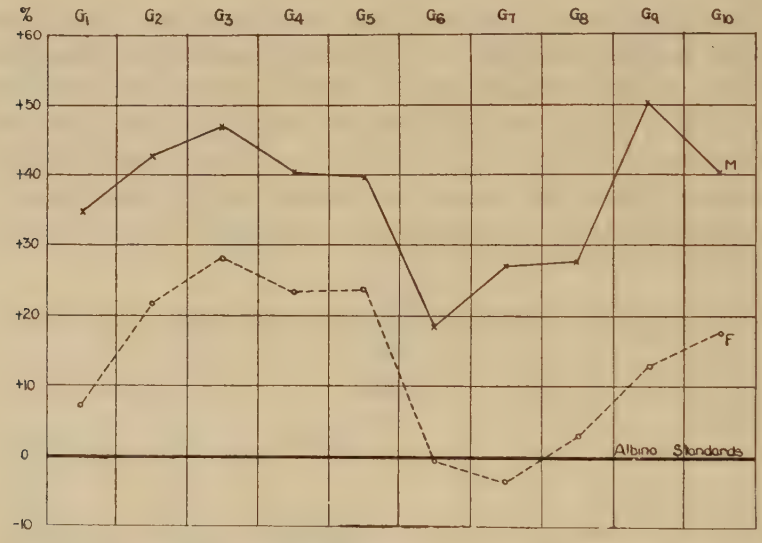

Chart 20 Weight of suprarenals in captive Grays. Percentage deviations in weight of suprarenals on body length, both sexes—Albino standards.

the graphs shows similar trends in the deviations for the two sexes. The sag from G_6 to G_8 is very marked, but neglecting this there is no tendency for a progressive diminution in the weight of the suprarenals as between the earlier and later generations.

It should be noted that, in the case of the Albino, the weight of the suprarenals in the female exceeds that in the male by about 90 per cent for the body lengths here considered. In the case of the captive Grays, however, the female suprarenals are in excess by only about 41 per cent. When, therefore, the deviations based on the albino standards for each sex are plotted on the same chart, as is here done, the graph for the female necessarily runs below that for the male.

To determine the difference in relations between the captive Grays and wild gray Norways, the data for the former have been compared with those given in *table 206*. Such a comparison shows that in the captive Grays the weights of the suprarenals are, on the average, less than in the wild strain by some 21 per cent in the males and 32 per cent in the females. The suprarenals in the captive Grays have thus lost weight as compared with those in the wild strain, and the greater loss is shown by the females.

We conclude, therefore, that captivity has reduced the weight of the suprarenals in the captive Grays, and that this reduction is more marked in the female. Such reductions represent a shift toward the weight values for the Albino. The weight relations according to sex are, however, brought closer in the captive Grays, and hence, away from the relation found both in the Albino and in the wild Gray. Thus the female suprarenals appear to be more responsive than those of the male. Finally, after the distinct drop between the values for the wild form and G_1, the graphs in chart 20 do not show a progressive diminution in weight from G_1 to G_{10}, but they do show a high degree of response to unfavorable conditions from G_6 to G_8—a feature of some importance.

TABLE 22

Suprarenal weights (both glands) in the captive Grays and deviations from the corresponding standards on body weight and body length. (Chart 20.) Albino standards. Males

GENERATION	NUMBER OF CASES	OBSERVED WEIGHT	ON BODY WEIGHT	ON BODY LENGTH	PERCENTAGE DEVIATION	
					On body weight	On body length
G_1	23	.0707	.0501	.0525	+ 41.1	+ 34.7
G_2	40	.0736	.0488	.0517	+ 50.8	+ 42.4
G_3	17	.0766	.0523	.0521	+ 46.5	+ 47.0
G_4	27	.0786	.0530	.0561	+ 48.3	+ 40.1
G_5	20	.0700	.0491	.0501	+ 42.6	+ 39.7
G_6	20	.0662	.0524	.0560	+ 26.3	+ 18.2
G_7	23	.0701	.0528	.0552	+ 32.8	+ 27.0
G_8	21	.0650	.0505	.0510	+ 28.7	+ 27.5
G_9	20	.0754	.0527	.0501	+ 43.1	+ 50.5
G_{10}	20	.0727	.0517	.0519	+ 40.6	+ 40.1
					Average:	+ 36.72

TABLE 23

Suprarenal weights (both glands) in the captive Grays and deviations from the corresponding standards on body weight and body length. (Chart 20.) Albino standards. Females

GENERATION	NUMBER OF CASES	OBSERVED WEIGHT	ON BODY WEIGHT	ON BODY LENGTH	PERCENTAGE DEVIATION	
					On body weight	On body length
G_1	18	.0888	.0695	.0832	+ 27.8	+ 6.8
G_2	26	.0881	.0652	.0727	+ 34.8	+ 21.6
G_3	26	.1002	.0692	.0783	+ 44.8	+ 28.1
G_4	24	.0997	.0692	.0810	+ 44.1	+ 23.1
G_5	18	.0947	.0708	.0761	+ 33.8	+ 23.5
G_6	18	.0917	.0828	.0925	+ 10.8	— 0.9
G_7	21	.0789	.0735	.0821	+ 7.4	— 3.9
G_8	22	.0857	.0778	.0832	+ 10.2	+ 3.0
G_9	20	.0892	.0763	.0791	+ 16.8	+ 12.8
G_{10}	21	.0920	.0771	.0783	+ 19.3	+ 17.5
					Average:	+ 13.64

WEIGHT OF GONADS IN THE CAPTIVE GRAYS

Standards: *Reference table 146* for the Albino and *table 207* for the wild gray Norway. In both sexes the gonads in the wild gray Norway are heavier than in the Albino.

The percentage deviations in the weights of the gonads, on albino standards, are given in table 24. The corresponding graphs, on body length, are entered in chart 21.

TABLE 24

Weight of gonads in the captive Grays and deviations from corresponding standards on body weight and body length. (Chart 21.) Albino standards

GENERATION	NUMBER OF CASES	STANDARDS			Percentage deviations	
		Observed	On body weight	On body length	On body weight	On body length
Testes						
G_4	27	3.548	2.985	3.093	18.9	14.7
G_5	20	3.013	2.841	2.825	6.1	6.7
G_6	20	3.441	2.969	3.092	15.9	11.3
G_7	23	3.354	2.981	3.063	12.5	9.5
G_8	21	3.715	2.899	2.916	28.2	27.4
G_9	20	3.458	2.980	2.884	16.0	19.9
G_{10}	20	3.894	2.963	2.945	31.4	32.2
					Average:	+ 17.4
Ovaries						
G_4	24	.0985	.0505	.0515	95.1	91.3
G_5	18	.1017	.0506	.0510	101.0	99.4
G_6	18	.1173	.0515	.0520	127.8	125.6
G_7	21	.0968	.0509	.0515	90.2	88.0
G_8	22	.1119	.0512	.0515	118.6	117.3
G_9	19	.0953	.0513	.0515	85.8	85.1
G_{10}	21	.1024	.0512	.0512	100.0	100.0
					Average:	+ 100.9

The records do not begin until G_4. As shown in chart 21, the graphs for the two sexes exhibit the same trends, save at G_5. This suggests that the influences affecting the weight of the gonads act in a similar manner in both sexes.

As compared with the albino standard, the data for the captive Grays average 17 per cent higher for the testes and 101 per cent higher for the ovaries.

A comparison of the data for the wild strain, *table 207*, with those for the captive Grays indicates that these latter run about 1 per cent higher in the male and 8 per cent higher in the female. No significance is to be attached to these dif-

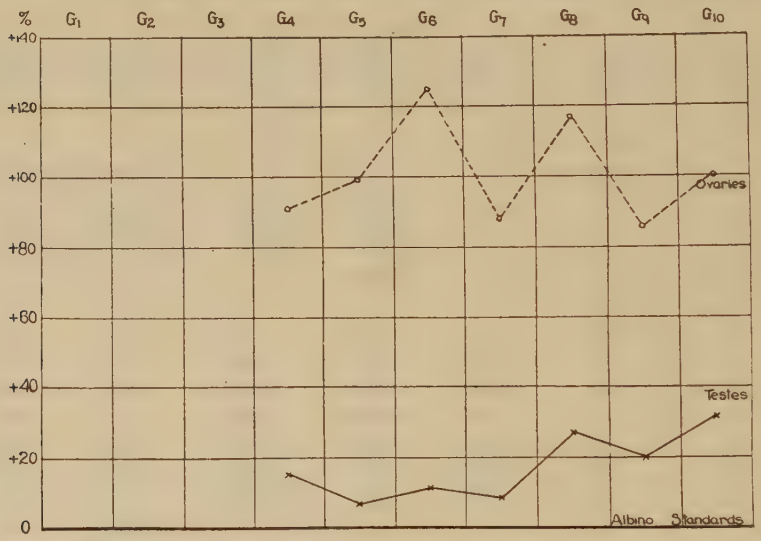

Chart 21 Percentage deviations in the weight of the gonads in captive Grays: Ovaries: testes. Albino standards.

ferences in themselves, but they serve to indicate that no important modification has been made in the weights of the gonads. Thus, from G_4 to G_{10} the gonads remain as in the wild strain and, therefore, show no tendency to assume the albino values which run much lower in both sexes. Nevertheless, while there is no evidence for the influence of the unfavorable conditions on the weight of the testes, there is perhaps some evidence at G_5, G_6, and G_7 for a depression in the weight of the ovaries.

PART II, SERIES 2. ON THE PARTS OF THE SKELETON IN THE CAPTIVE GRAYS

Before discussing the effects of captivity, as just presented in series 1, the observations on the parts of the skeleton, series 2, will be considered.

The rats used for this series were from the same generation groups as those examined in series 1. They were, however, different individuals, and the data do not become available until G_4.

With two exceptions, the averages used were based on five individuals in each sex group in each generation. The dissections were all by Miss Ruth Meeser. Using this material, the following determinations have been made: 1) the weight; 2) the length, and, 3) the percentage of water for the cranium, mandible, humerus, and femur.

The purpose of these observations was to determine whether there were any progressive changes taking place in the bones of the captive Grays between G_4 and G_{10} and also to determine whether such changes indicated a shift toward the values for the Albino. In the absence of corresponding observations on the wild strain, the relations of the captive Grays to this strain could not be determined.

The relations found have proved to be so similar in all the four parts of the skeleton examined that the data for the femur, which are typical, will alone be presented here.

The standard values used for the several base lines are from *reference tables 131, 128,* and *163* for the Albino. The computed deviations are given in table 25 and plotted in chart 22.

Examination of the graphs shows that the deviations in the length of the femur are slight and those that occur are mainly retardations in response to unfavorable conditions. The deviations in weight are, however, large, and the retarda-

tions at G_5, G_6, and G_7 are clearly marked. At the same time, the deviations in the percentage of water follow the deviations in weight. On considering these changes together, it appears that when the femur is less in weight the medullary cavity also becomes less, and as this cavity carries water-bearing materials the percentage of water is thereby diminished. The deviation in the percentage of water in this case

TABLE 25

Giving the percentage deviations for the weight, length, and percentage of water in the femur of the captive Grays

GENERATION	SEX	AGE	BODY LENGTH	PERCENTAGE DEVIATIONS		
				Weight (fresh)	Length (fresh)	Per cent water
			Mm.			
G_4	M	523	233	+ 29.3	+ 0.3	+ 14.1
	F	516	222	+ 33.6	— 1.0	+ 13.0
G_5	M	590	234	+ 20.0	— 5.0	+ 0.9
	F	602	230	+ 12.1	— 2.0	— 0.3
G_6	M	559	251	+ 26.1	— 1.3	— 0.3
	F	599	231	+ 14.8	— 3.0	— 3.1
G_7	M	583	235	+ 22.5	— 3.0	— 0.05
	F	606	218	+ 14.8	— 8.0	+ 5.4
G_8	M	578	242	+ 34.5	+ 2.0	+ 7.5
	F	592	226	+ 23.3	+ 2.0	+ 8.9
G_9	M	606	230	+ 38.7	— 1.4	+ 6.9
	F	601	222	+ 31.2	— 2.0	— 4.0
G_{10}	M	598	229	+ 33.4	— 0.2	— 4.9
	F	591	223	+ 31.7	— 2.1	+ 2.5

is, therefore, not to be referred to the osseous tissue in the strict sense, but to the entire bone as an organ.

There is one feature in these graphs that calls for comment, namely, the frequency of low values at G_5. In series 1 the first low value marking a sag in the graph usually comes at G_6. We infer from this relation that the unfavorable conditions which are assumed to produce these retardations are effective in modifying the skeleton in animals that are older than those in which the several organs are affected (chart 1).

It appears, therefore, that between G_4 and G_{10} the various parts of the skeleton show no progressive changes in the characters measured. Whether there has been a lowering of values previous to G_4 our data do not indicate. These parts of the skeleton do show retardations under unfavorable conditions, and the response can apparently be obtained in rats that are younger than those that show organ changes.

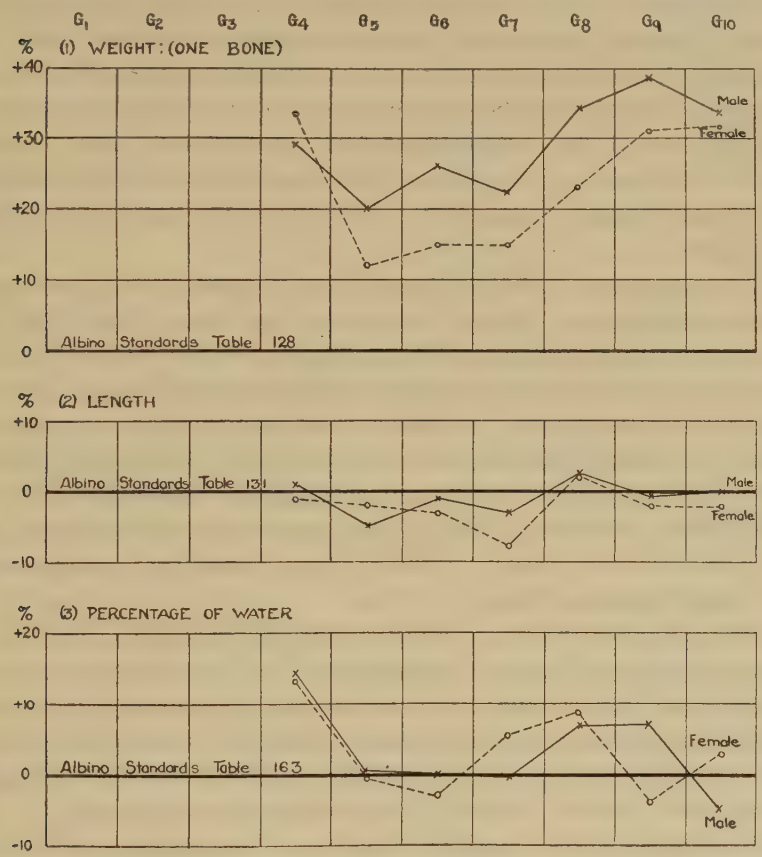

Chart 22 Data on femur: Percentage deviations on body length in captive Grays of, 1) weight; 2) length; 3) percentage of water—Albino standards.

DISCUSSION

Attention is called to the fact that the data here to be considered are from the first ten generations only of gray Norway rats in captivity. To obtain the full effects of captivity on the body and its organs will probably require many more generations, and, therefore, any conclusions drawn from the present data must be largely tentative.

Body size. This is best measured by body length, and chart 12 gives the graph for the observed values. As the entries in tables 11 and 12 show, the body length at G_1 is for rats much over 600 days of age and at G_2 for those under this age, while the remaining eight entries run close to 600 days. Correction for age would bring down the body length at G_1 and slightly raise it at G_2. Both changes are small, however.

If the graphs for body length are viewed with these possible corrections in mind, it is seen that the values for the first three generations are low, but, in both sexes, giving average values not essentially different from those for the last three generations. Moreover, the unfavorable conditions which were acting from G_5 to G_8 do not produce any clear-cut effects as indicated by these graphs. The mean body-length value for the series is 240 mm. for the males and, for the females, 223 mm. Captivity has, therefore, not altered the *body length on age.*

Body weight. In chart 13 the relations of the body weights as observed to those to be expected on the observed body length (*reference table 187*) are shown by the graphs. The graphs also show that the observed values exceed the tabular values for the wild gray Norways and that the excess tends to increase in the successive generations, so that at G_{10} the body weights of the captive Grays are in excess by 16.6 per cent in the males and by 16.2 per cent in the females. This excess in body weight is similar to that shown by the Albinos, so that captivity for ten generations has developed in the captive Grays the weight-length relations which are characteristic for the Albino. However, the graphs sag slightly

at G_6 and G_7 in response to the unfavorable conditions then acting.

To summarize the foregoing: In captivity for ten generations the gray rat shows a constant body length on age, but a weight-length relation which differs from that for the wild Norway, and agrees at G_{10} with that for the Albino. These captive Grays, then, are rats which grow normally in length, but tend to become fatter as the generations progress.

In these captive Grays the weights of the brain, hypophysis, thyroid, and suprarenals have been followed from G_1 to G_{10}.

Brain weight. As to these values in the ancestors of this series little can be said. The brain weight for the male ancestors was that expected for the wild gray Norways, *reference table 187,* but for the female ancestors it was 2.5 per cent low. The G_1 values are definitely lower, as shown in chart 14. This drop at G_1 is followed by a recovery in weight, which runs into a sag at G_7 and G_8 and then again recovers. On the average, both sexes show brains 5 per cent or less below the standard for the wild gray Norway, but within the ten generations there has been no progressive diminution in weight. It appears, therefore, that the conditions of captivity cause an immediate drop (G_1) in brain weight, but within ten generations this drop does not further increase.

Hypophysis weight. When compared with the data for the wild strain, the observations on the captive Grays show a tendency to an increase in weight, the values for the males running 7 per cent and for the females 11 per cent above those for the wild strain. Thus, although there is a sag in the graphs at G_6 and G_7 and no progressive change in weight, there appears to be in the hypophysis a slight increase in weight as the result of captivity. So far as this goes it is a shift toward the relations in the Albino, but it does not go far enough to produce the very heavy female hypophysis characteristic of the Albino.

Thyroid weight. The thyroid gland is the only organ thus far found in which there is a progressive loss in weight. In

G_1 the weight is already reduced below the reference-table value, and the reduction, with a slight intensification at G_6 and G_7, continues to G_{10}. This weight change is certainly an effect of captivity in the broad sense, but is most probably due to a special condition represented by the food, and not to the more general conditions surrounding the captive animal. In this sense the response of the thyroid seems a bit peculiar and different from those made by the other organs. At G_{10} the thyroid in the captive Grays has weights close to those now current in this laboratory for the Albino. Structural defects in the thyroid and in the parathyroid were also noted (table 21).

Suprarenal weights. Already at G_1 the weight of the suprarenals is low. Then follows a recovery in weight which is interrupted by a retardation of growth at G_6, G_7, and G_8, to be once more followed by a recovery that reaches about the initial values. Thus, there is no progressive change in weight. As compared with the wild strain, the captive Grays have distinctly smaller suprarenals in both sexes, thus approaching the relations in the Albino, but the weight relations according to sex are shifted away from those in the Albino, since the weights according to sex come nearer together. The suprarenals are conspicuous, however, in the fullness of their response to unfavorable conditions. They apparently act as a sort of physiological barometer, registering directly the pressure of unfavorable conditions by a retardation in their growth.

This completes the examination of the weight changes in the four organs studied from G_1 to G_{10}. There remain the gonads and the parts of the skeleton (series 2) for which data are available only from G_4 to G_{10}. Obviously, in these cases nothing can be said about the initial drop in weight at G_1, and it is possible to compare only the G_4 with the final values and to record any indication of the effect of unfavorable conditions.

Gonads: weight. The graphs in chart 21 show that in the testes the G_4 and final weights are similar. There is no

indication of the influence of unfavorable conditions, and the weights for the captive Grays are similar to those for the wild strain. Thus, there is no modification in the weights bringing these down to those of the Albino. On the whole, captivity and unfavorable conditions appear to have produced less effect on the testes than on any other organ studied.

In the case of the ovaries, the weights remain as high or even a little higher than in the wild strain, but in contrast to the testes the ovaries do seem to respond to the unfavorable conditions with lower values at G_5 and G_7. The failure of the gonads to change in weight during the first ten generations of captivity was unexpected, in view of the much lower weight values for the gonads shown by the Albinos.

Parts of the skeleton (series 2), table 25, chart 22

Length, weight, and percentage of water. Femur. As noted in the previous consideration of the data in series 2, the various parts and bones examined for length, weight, and percentage of water agree in their general behavior with one another. For this reason it was deemed sufficient to note in detail the results for the femur. Between G_4 and G_{10} there is, in the femur, no progressive change in length, weight, or percentage of water. The unfavorable conditions are marked by low values in each of these characters from G_5 to G_7. We have no reference tables for these characters in the wild strain, but, as the results stand, the femur in the captive Gray is distinctly heavier and has about the same length and a slightly greater percentage of water than in the Albino. Under unfavorable conditions, the femur becomes more slender (i.e., the weight is more reduced than the length), the cavity is diminished, and the percentage of water decreased. All that can be said in a general way is that there is no progressive change in these characters between G_4 and G_{10}. The striking result in this case is the great susceptibility of the osseous system—as represented by the femur—to unfavorable conditions, to which it responds with marked reductions in weight and in percentage of water.

Using the data for the wild gray rat as standards, the effects of captivity for ten generations on the gray Norway rat, as shown by the foregoing data, may be formulated as follows:

1. Body length on age is not modified.

2. Body weight on body length increases progressively and at G_{10} has the relation found in the Albino.

3. In the organs:

a. An initial drop in weight (ancestors to G_1) apparently occurs in the brain, thyroid, and suprarenals. It cannot be determined in the gonads or bones, as the G_1 values are not available.

b. Progressive loss in weight is shown by the thyroid—the only organ behaving this way. This is probably due to the character of the food.

c. A slight increase in weight is shown by the hypophysis.

d. No progressive loss in weight between G_1 (or G_4) and G_{10} occurs in the brain, hypophysis, suprarenals, gonads, or bones.

e. A shift in the weight values toward those characteristic for the Albino is shown by the brain, hypophysis, thyroid, and suprarenals. This point cannot be determined in the case of the bones.

4. Unfavorable conditions, represented by louse infection, delousing, high temperature, and poor care (chart 1), are associated with low values in the graphs, except in the cases of body length and of the testes. Table 26 gives the generations in which low values appear in the several instances. In the seven cases in which a response occurs it always appears at G_7 and, with the exception of the brain, always at G_6, but in two instances, the ovaries and the bones, it is seen also at G_5 and in another two instances, brain and suprarenals, at G_8. The interpretation of the two extreme groups is that, in the case of the ovaries and the bones, unfavorable conditions can modify the weight even after growth is well advanced, while in the case of the brain and suprarenals the modification is either through the young themselves or even through effects on the parents.

5. Sex differences in weight.

In the Albino, sex differences in weight are conspicuous in the hypophysis and suprarenals, the gland in the female being much heavier in each case.

In the captive Gray:

Hypophysis: The sex weight relation changes slightly, giving a somewhat heavier hypophysis in the female captive Gray. This is the Albino relation.

TABLE 26

To show the generations in which low values occur in the several instances

	GENERATION			
	G_5	G_6	G_7	G_8
Body weight		X	X	
Body length				
Brain			X	X
Hypophysis		X	X	
Thyroid		X	X	
Suprarenals		X	X	X
Testes				
Ovaries	X	X	X	
Femur	X	X	X	

X indicates a low value.

Suprarenals: The weight of the female gland is brought closer to that for the male. This is a shift away from the relations for both the wild Norway and the Albino.

6. All these determinations have been made on animals approximately 600 days old (equivalent to 50 years in human age). The values studied are terminal values, and it is possible to determine only indirectly, as by the effect of unfavorable conditions, at what phase of growth the various modifications occurred.

7. The larger results are these: In only one organ, the thyroid, is there a progressive loss in weight, and this is most probably due to food. One organ, the hypophysis, increases

in weight slightly. Unfavorable conditions produce organs of smaller weight, but though this response takes place, the body length remains unchanged and the body weight is but slightly affected.

CONCLUSIONS

After ten generations in captivity, the captive Gray differs from the wild type as follows:

There is an increase in body weight on body length. A slight increase in the weight of the hypophysis. No change occurs in the weights of the gonads. Less in weight are the brain, the thyroid, and the suprarenals, especially the female suprarenals.

In organs of lesser weight the greater loss occurs at G_1, but there is no subsequent progressive loss, save in the thyroid.

Similarly, the captive Gray compared with the Albino shows the following relations:

The brain, the suprarenals (especially in the male), the gonads, and the bones are still heavy. Body weight on body length and the weight of the thyroid are similar to those for the Albino.

The hypophysis, especially that of the female, remains less in weight.

Ten generations of captivity have, by no means, served to give the captive Grays the organ constitution of the Albino.

In view of the preliminary character of this report, no survey of the literature will be attempted at this time.

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THE AMERICAN ANATOMICAL MEMOIRS

ON THE PROBLEM OF LYMPH FLOW BETWEEN CAPILLARIES OF THE BLOOD-VASCULAR SYSTEM AND BLINDLY-ENDING CAPILLARIES OF THE LYMPHATICS

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TO MY WIFE

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ON THE PROBLEM OF LYMPH FLOW BETWEEN CAPILLARIES OF THE BLOOD-VASCULAR SYSTEM AND BLINDLY-ENDING CAPILLARIES OF THE LYMPHATICS

I. INTRODUCTION

During the last twenty years, the writer has been specially interested in the morphology and the physiology of the lymphatic system. In the course of his investigations several problems have presented themselves. Among these is an inquiry into the causes of oedema, concerning which two papers have already been published.[1] Another problem concerned a possible provisional arrangement of the embryonic lymphatic system, by which could be explained the manner in which a centripetal lymph flow toward the venous circulation from the interstitial spaces could be controlled and regulated until the lymph could reach the venous circulation.

It is now well known that the anlagen of the lymphatic system do not make their appearance in the embryo until after the blood-vascular channels have been established. As soon, however, as the latter begin to function, lymph necessarily begins to collect in the interstitial spaces of the embryo, and accumulates there until it is subsequently collected by the newly formed set of blindly ending vessels, the lymphatics, by which it is conveyed to the venous circulation.

One of the salient facts observed in the development of the vascular system is that the main channels, both haemal and lymphatic, develop *in loco* from mesenchyme and are formed

[1] McClure, C. F. W. 1919 On the experimental production of oedema in larval and adult anura. Jour. Gen. Physiol., vol. I.

———— 1925 An experimental analysis of oedema in the frog, with especial reference to the oedema in red-leg disease. Amer. Anat. Mem., no. 12.

through the concrescence of independent and discontinuous anlagen. See McClure, 1921, for an historical account of the investigations bearing on this subject.[2]

A study of living trout embryos made by the writer[3] has demonstrated that certain of these independent anlagen of the lymphatic system, the subocular lymph sacs, receive through their closed endothelial-lined walls lymph which flows from the interstitial spaces in a centripetal direction, and that the lymph received in these sacs is temporarily retained by them until the sacs secondarily join the lateral pharyngeal lymphatic (jugular), by which it is then transported to the venous circulation. Subocular lymph sacs were observed also in the embryos of Amia and Lepidosteus. In the embryos of ganoids, however, these sacs communicated directly with the veins at a very early stage of development, so that the lymph received in them was not retained, but was directly transported to the venous circulation. What we find to be the case in both trout and ganoids is that lymph from the tissue spaces flows in a centripetal direction through the endothelial-lined walls of blindly ending lymphatics, and that in the case of the trout this lymph is temporarily retained by closed and detached independent anlagen of the lymphatic system, until the conditions are such that the centripetal flow can be continued to the venous circulation. It can therefore actually be demonstrated in the living trout embryos not only that certain lymphatics end blindly, but also that certain of the independent anlagen of the lymphatic system serve as reservoirs for the reception and temporary retention of lymph received from the interstitial spaces prior to the estab-

[2] McClure, C. F. W. 1921 The endothelial problem. President's address: read before the American Association of Anatomists in Philadelphia. Anat. Rec., vol. 22.

[3] McClure, C. F. W. 1915 The development of the lymphatic system in fishes with especial reference to its development in the trout. Amer. Anat. Mem., no. 4.

———— 1915 On the provisional arrangement of the embryonic lymphatic system. An arrangement by means of which a centripetal lymph flow toward the venous circulation is controlled in an orderly and uniform manner, from the time lymph begins to collect in the intercellular spaces until it is forwarded to the venous circulation. Anat. Rec., vol. 9.

lishment of continuous lymphatic channels, by which it is subsequently conveyed to the venous circulation. To what extent independent endothelial-lined and closed lymph sacs of the embryo, other than the subocular sacs of the trout, function in a similar manner during ontogeny, is a question still to be determined. In any event, we can now demonstrate in the living trout embryo that lymph in the interstitial spaces received from the blood capillaries collects in certain closed lymph sacs which are at first independent of the veins and of other lymphatics.

It was largely through these researches on the development of the lymphatic system that the writer was led to study the problem of the flow of lymph between the blood capillaries and the lymphatics; and his investigation is set forth in the following pages. The study concerns chiefly the capacity of frog skin (whether it be the skin of the living animal or skin detached from the body) to transport water through itself under various experimental conditions. Many observations pertinent to the present study have been made by previous writers; the main feature of the present work is its analysis of an extended series of observations, which indicates a possible explanation of the continuous flow of fluid between the blood capillaries and the blindly ending capillaries of the lymphatics.

We know that water is chiefly supplied to the body of the living frog by osmosis through the integument into the subcutaneous lymph sinuses. Reid,[4] Overton,[5] and Maxwell[6] have observed that when a skin glove, with the outside of the skin turned outward, is filled with a Ringer's solution and suspended in the same solution, water may, in spite of the equilibrium of osmotic pressure, be transported through the skin into the glove.

[4] Reid, E. W. 1890 Osmosis experiments with living and dead membranes. Jour. Physiol., vol. XI.

[5] Overton, E. 1904 Neununddreissig Thesen über die Wasserökonomie der Amphibien und die osmotischen Eigenschaften der Amphibienhaut. Verhl. d. Phys.-med. Ges. zu Würzburg, N.F., Bd. 36.

[6] Maxwell, S. S. 1913 On the absorption of water by the skin of the frog. Amer. Jour. Physiol., vol. 32.

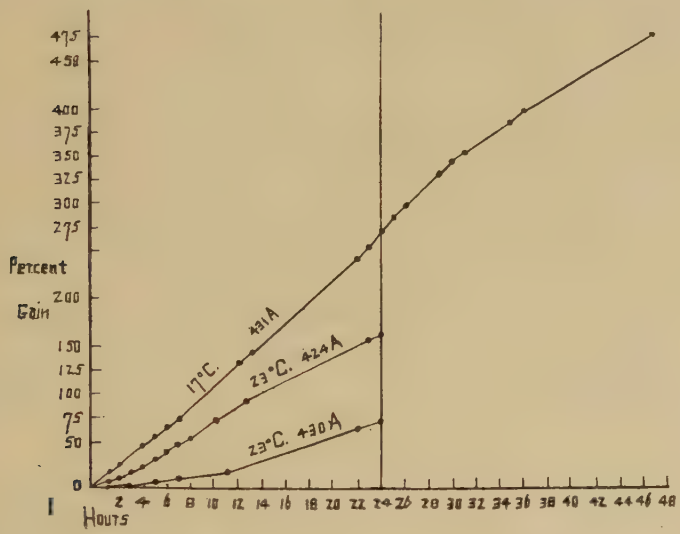
It has often been stated that the skin of frogs of the same as well as of different species may vary greatly in its general character, especially as regards permeability. Since we now recognize the fact that water is transported through the skin into the glove when the latter, with the outside of the skin turned outward, is filled with a Ringer's solution and is suspended in the same solution, it seems probable that a study of frog skins, as regards their relative permeability, might throw a new light on still other phenomena associated with the transport of fluid through the skin.

The technique employed in the preparation of the skin gloves used in this investigation was essentially the same as that employed by other investigators. In most cases the skin of the hind legs was used in the form of a glove, a ligature being applied to the opening left where the skin had been cut off at the ankle. The upper end of the glove was tied to a glass tube, which was then closed by a paraffined cork to prevent evaporation of fluid placed in the glove when the latter was suspended in solutions. This method of preparation allows the experimenter to change the fluid in the glove, and so to make use of the same glove for different experiments. At stated intervals the gloves were removed from the solutions and were weighed. Always the outside of the skin was dried rapidly with filter-paper before the weighing was done. The experimental error involved in weighing skin gloves obtained from the hind legs of small frogs amounted to about 5 milligrams.

II. THE BEHAVIOR OF GLOVES OF FROG'S SKIN, WITH THE *OUTSIDE* OF THE SKIN TURNED *OUTWARD*, WHEN FILLED WITH RINGER'S SOLUTION APPROXIMATELY ISOTONIC WITH FROG'S BLOOD, AND SUSPENDED IN THE SAME SOLUTION

The writer has made a large number of these experiments. They were made in every month of the year; the skins used were taken from living frogs which had been recently captured, and also from frogs which remained in captivity without food for several months prior to the experiments. The normal average behavior of the skin gloves has been graphi-

cally represented in figures 1, 2, 3, and 4. *In these experiments, and in all others of a similar sort, the percentage of the gain or the loss in weight of the skin glove, unless otherwise stated, has been estimated upon the original weight of the empty skin glove at the time of its removal from the body—that is, before fluid was placed in the glove and before the glove was suspended in the solution. For example, in experiment 431 A (fig. 1) a gain in weight of the skin glove amounting to about 475 per cent indicates that during a period of forty-eight hours the weight of the fluid trans-*



ported through the skin into the glove amounted to 475 per cent of the weight of the empty skin glove before it was filled with Ringer's solution and before it was suspended in the solution.

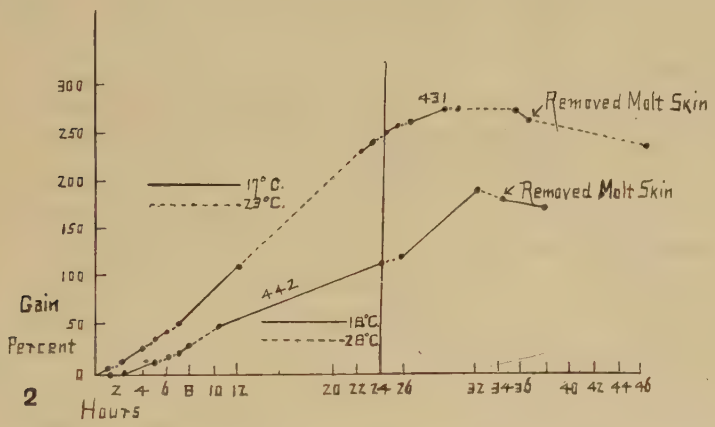
Figure 1 represents the range of behavior observed in the case of three skin gloves removed from the hind legs of three different healthy frogs (R. pipiens) which had been supplied by a dealer about one month prior to the experiments. Each skin glove, with the outside of the skin turned outward, was filled with Ringer's solution approximately isotonic with frog's blood, and was suspended at a *constant* temperature in

the same solution. It will be observed that, during a period of twenty-four hours' suspension in the solution, these three gloves showed marked differences in the rate at which water was transported through the skin from without inward. In one case the gain in weight of the glove amounted to 272.2 per cent; in the two remaining gloves it amounted to 160.7 and 68 per cent, respectively. In cases where the rate of passage of water through the skin was relatively rapid, it was found that the glove continued to gain in weight for a period of forty-eight hours or more, although the gain was less on the second than on the first day of suspension. These three experiments showed that, among different frogs of the same species, during the same season of the year, marked differences exist in the rate at which water is transported through the skin into the glove under the conditions above mentioned.

When skin gloves, with the outside of the skin turned outward, are filled with Ringer's solution approximately isotonic with frog's blood and are alternately suspended in the same solution at *different* temperatures, their behavior may be the same during the first day's suspension as if the temperature of the Ringer's solution had been constant (experiments 431 and 442, fig. 2). Also, the rate at which water is transported through the skin into the glove is not invariably influenced by temperature, in the sense that the gain in weight of the glove is greater at a high than at a lower temperature. As a matter of fact, instances were frequently met with in which the reverse conditions were observed.

During the second day of their suspension in Ringer's solution at different temperatures, the skin gloves usually showed signs of molting, and the rate at which water was then transported through the skin into the glove became greatly diminished. After the loss of the superficial layer of skin (molt skin), a complete reversal was brought about in the direction in which the transport of water through the skin was accomplished. In such cases water was transported through the skin *outward* instead of *inward*. The behavior of skin gloves during the second day of their suspension in

isotonic Ringer's solution and after molting is shown in figure 2. In experiments 431 and 442 (fig. 2) the molt skin was removed from the glove at the time indicated in the figure, after which the gloves began to lose in weight continuously. A comparison of experiment 431 A (fig. 1) with experiment 442 (fig. 2) illustrates the behavior of two skin gloves taken from the same frog when filled with isotonic Ringer's solution and suspended in the same solution at a constant (431 A) and at different (442) temperatures. The time of molting naturally varies greatly in different cases, and is largely influenced by the temperature of the solution



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in which the glove is suspended. At the time of their removal from the body, skins in which the rate of transport of water is relatively slight show signs of molting earlier than those in which the rate is higher.

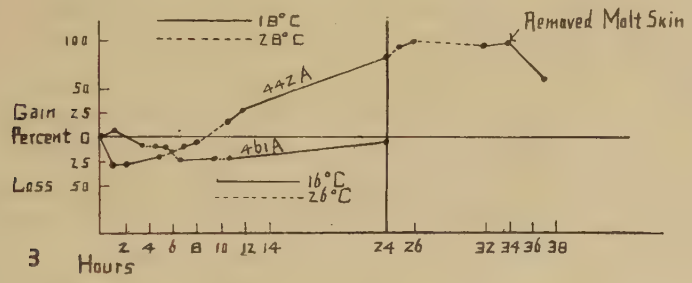
In most cases skin gloves, with the outside of the skin turned outward, filled with isotonic Ringer's solution, immediately begin to gain in weight and continue to do so for a considerable time, either at a constant or at varying temperatures, when suspended in the same solution. Exceptions to this behavior are, however, found. In some instances a loss in weight, instead of a gain, immediately succeeds the suspension of the glove in Ringer—though such temporary loss is almost invariably succeeded by a gain. This form of be-

havior is illustrated by experiment 442 A (fig. 3), in which the initial loss in weight of the glove amounted to 25 per cent during the first hour of its suspension in Ringer; then a gain in weight followed up to the twenty-sixth hour, when the weight remained stationary until the molt skin was removed from the glove. The behavior of the glove in experiment 461 A (fig. 3) differs slightly from that observed in the last-mentioned experiment. In experiment 461 A a very slight gain in the weight of the glove, during the first hour of its suspension in Ringer, was succeeded by a loss which continued for five hours, after which there was a slight gain. The two frogs from which these skins were taken were very thin and weak, and, in experiment 461 A, the frog used was quite emaciated.

In figure 4 is graphically shown another experiment (437) made on a skin glove removed from the hind leg of a frog (R. pipiens) which was thin and which had molted just prior to the removal of the skin from the body. This experiment is interesting as showing the influence of temperature on the weight of a glove with the outside of the skin turned outward, filled with Ringer's solution approximately isotonic with frog's blood and alternately suspended in the same solution at different temperatures. During the first five hours of its suspension in Ringer at 17°C., the glove either gained or remained stationary in weight; when transferred to the same solution at 27°C., however, the glove lost. Between the fifth and twentieth hours, the gain in weight of the glove was continuous, irrespective of differences in temperature; after this, however, in relation to temperature changes, the weight showed fluctuations similar to those of the first five hours. While this type of behavior does not appear to be especially common, a sufficiently large number of cases has been observed to justify placing it among the typical forms which one occasionally encounters. Perhaps the most important and significant feature of this last-mentioned behavior is that exactly similar fluctuations in body weight can be produced in the living frog when the latter is transferred from water at one temperature to water at another.

III. THE BEHAVIOR OF HEALTHY LIVING FROGS WHEN TRANSFERRED FROM WATER AT ONE TEMPERATURE TO WATER AT ANOTHER

In one hundred experiments made by the writer, in which normal healthy frogs were transferred from water at a low to water at a higher temperature, seventy-three lost and twenty-seven gained weight immediately after the transfer. The average initial maximum loss in weight amounted to 5.9 per cent, and the average maximum gain to 3.22 per cent.



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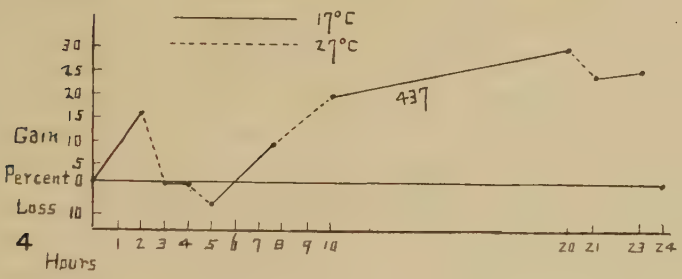

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Also, in sixty-six experiments, in which normal healthy frogs were transferred from water at a high to water at a lower temperature, fifty-six, or about 85 per cent, gained, and ten, or about 15 per cent, lost weight immediately after the transfer. The average initial maximum gain in weight amounted to 3.79 per cent and the average initial maximum loss to 2.44 per cent.

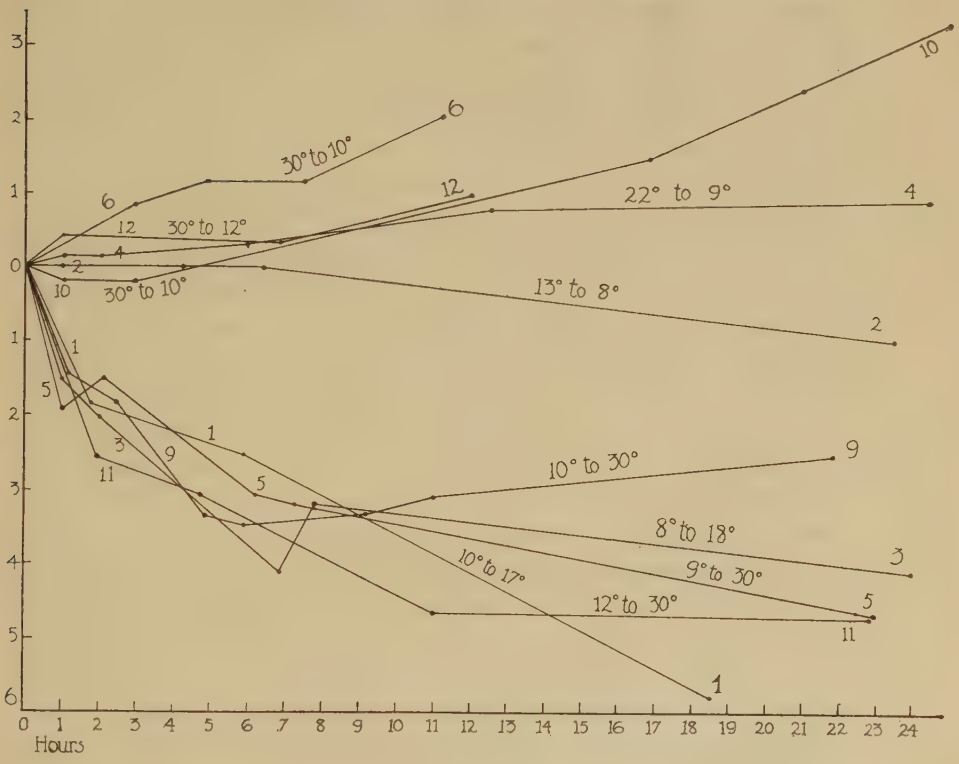
The fluctuations in body weight which resulted when a living frog (R. pipiens, no. 246) was alternately transferred from water at one temperature to water at another are graphically represented in figure 5. The numerals opposite

each curve in figure 5 indicate the order in which the experiments were made. For example, the first transfer (curve *1*) was made from water at 10°C., in which the frog had previously remained for a considerable time, to water at 17°C. The second transfer (curve *2*) was made from water at 13°C. to water at 8°C., and so on. Between experiments 6 and 9 two desiccation recovery curves (McClure, '25, fig. 1), were made, based on the reactions of the same frog. These are not included in the figure. The odd numbers (curves *1, 3, 5, 9,* and *11* in fig. 5) represent the behavior of the frog when transferred from water at a *low* to water at a *higher* temperature. The even numbers (curves *2, 4, 6, 10,* and *12* in fig. 5) represent the behavior of the frog when transferred from water at a *high* to water at a *lower* temperature. Had it been practical to do so, the behavior of this frog would have been graphically represented in one curve. In the present case the zero point on the ordinate marks the beginning of each transfer. This series of experiments extended over a period between March 26th and April 26th, and was made on a frog obtained from a dealer in February. During the course of the last experiment (curve *12*) the frog contracted red-leg disease, and its gain in weight then became continuous (McClure, l.c., '25). This last experiment (curve *12*) extended over a period of 170 hours, at the end of which time the gain in weight of the frog amounted to 32.8 per cent—a gain not graphically represented in the figure. Since a continuous increase in body weight in the frog is coincident with the appearance of oedema, the writer deemed it worth while to make an analysis of these fluctuations observed both in the skin and in the living frog.

IV. THE BEHAVIOR OF GLOVES OF FROG'S SKIN, WITH THE *INSIDE* OF THE SKIN TURNED *OUTWARD*, WHEN FILLED WITH RINGER'S SOLUTION APPROXIMATELY ISOTONIC WITH FROG'S BLOOD AND SUSPENDED IN THE SAME SOLUTION

When skin gloves, with the *inside* of the skin turned *outward,* are filled with isotonic Ringer's solution and suspended in the same solution, there is, as numerous investigators have

observed, a general tendency for water to pass from the glove through the skin outward, in a direction exactly opposite to that which occurs when the outside of the skin is turned outward. This transport of water outward through the skin is usually not at first evident, for the reason that the glove almost invariably gains weight during the first hour of its suspension in Ringer's solution. This gain in weight



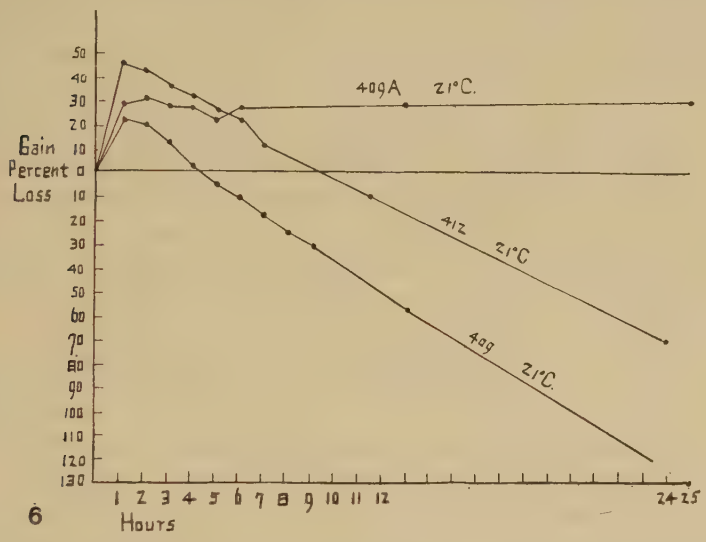
is not due, however, to the presence of water which has passed from without inward through the skin into the glove, but rather to the fact that the skin itself has absorbed and held water, in excess of that which is simultaneously transported from the glove through the skin outward. When the hydration capacity of the skin has been satisfied so that water is no longer absorbed by the skin itself, then the glove often begins to lose weight, due to a passage of water from the

glove through the skin outward. In other words, if the skin itself did not at first absorb and hold water, the glove would probably begin to lose weight immediately when suspended in Ringer's solution. It is therefore evident that at the start at least two separate factors acting in opposition to each other are involved in the behavior of skin gloves, with the *inside* of the skin turned *outward,* when filled with Ringer's solution isotonic with frog's blood and suspended in the same solution. One of these factors is the capacity of the skin itself to absorb and to hold water, and the other, the power of the skin to transport water through itself, when the skin is bathed on both surfaces by solutions having the same osmotic pressure. While both factors may act simultaneously, the action of one may mask or overbalance the other, so that, even though water is transported through the skin outward when the glove is first placed in Ringer's solution, the glove will not begin to lose weight until the hydration capacity of the skin has been satisfied and an equilibrium in this respect has been established—or, until the rate of absorption of water by the skin is exceeded by that at which water is simultaneously transported from the glove through the skin outward.

We have already seen that, when the outside of the skin is turned outward, the rate at which water is transported inward through the skin varies greatly in different cases (figs. 1, 2, 3, and 4). The same may be said concerning the rate at which water is transported outward through the skin when the inside of the skin is turned outward. We now pass to the consideration of a number of experiments which illustrate the behavior of skin gloves with the *inside* of the skin turned *outward,* when filled with Ringer's solution approximately isotonic with frog's blood and suspended in the same solution.

Experiments 409 and 412 (fig. 6) illustrate the behavior of skin gloves removed from the hind legs of two different frogs (R. pipiens) which had been kept in the laboratory during the winter prior to the experiments. The frogs were active, but their skin was pale and dull in color. In each

case, the glove, with the *inside* of the skin turned *outward,* was filled with isotonic Ringer's solution and suspended in the same solution at room temperature. At the end of the first hour of suspension, the gain in weight amounted in one glove (exper. 409) to 23.8 and in the other (exper. 412) to 47.5 per cent. After the first hour, however, each glove began to lose weight, through the transport of water from the glove through the skin outward. At the end of twenty-four hours' suspension in Ringer, the loss in one case amounted to 119



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(exper. 409) and in the other to 70.5 per cent (exper. 412). If we compare the behavior of these two gloves filled with Ringer's solution with that of an empty glove (exper. 409, A, fig. 6) taken from the hind leg of one of these same frogs (409), also with the *inside* of the skin turned *outward,* we observe that while the empty glove gained weight (31.6 per cent) during the first two hours of its suspension in Ringer's solution, after this time its weight remained practically stationary. In this instance the empty skin glove contained no fluid which could be transported from the glove through the skin outward and, since no fluid was transported through the

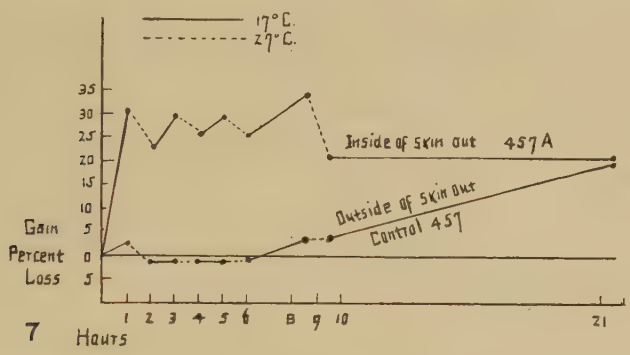
skin in the opposite direction, the gain in weight of the glove must have been due to the presence of fluid which had been absorbed by the skin and held by the latter. We have reason to suppose that if this same skin had been filled with Ringer's solution, its behavior would not have differed essentially from that of the glove which had been obtained from the same frog, but which had been filled with Ringer's solution (exper. 409).

When a living frog remains out of water, we know that as the result of loss of water from the body the osmotic pressure of its body fluid may be raised. The same may be said of skin gloves, which undoubtedly lose some water during the handling in preparation for the experiments. While osmosis may play some rôle in accounting for the fact that skin gloves, with the inside of the skin turned outward, almost invariably gain weight when first placed in Ringer's solution, the following experiments seem still further to bear out the view that this gain is due chiefly to the absorption of fluid by the skin until its tissue colloids have reached their hydration capacity.

Experiment 457 A (fig. 7) is one of interest for the reason that it shows a form of behavior indicating that a gain or a loss in weight of the glove is influenced by temperature, but cannot be explained on the grounds of osmotic pressure.

In this experiment a skin glove, with the *inside* of the skin turned *outward,* which had been removed from the right hind leg of a frog (R. pipiens) kept in the laboratory for several months prior to the experiment, was filled with Ringer's solution approximately isotonic with frog's blood and was alternately suspended in the same solution at 17°C. and at 27°C. During the first hour of its suspension at 17°C. the glove gained about 30 per cent, because the skin itself had a marked capacity to absorb and hold water. During a period of eight hours' suspension following this initial gain, the glove uniformly lost weight when transferred to Ringer's solution at the high temperature (27°C.) and gained weight when transferred from this solution to one at the low tem-

perature (17°C.). As previously stated, during the first hour the gain in weight of the glove may have resulted partly from differences in osmotic pressure of the fluid in the skin and that of the solution in which the glove was suspended; but it would be difficult to explain its succeeding behavior as an osmotic reaction. The loss in weight of the glove during the second hour indicates that the hydration capacity of the skin had previously been satisfied; and the fact that a loss in weight regularly occurred when the glove was suspended in Ringer at 27°C., and a gain when it was suspended in Ringer at 17°C., indicates that the gain and the loss in weight were due not to osmotic pressure, but rather to the gain and the loss of water by tissue colloids.



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The control of this experiment (457, fig. 7), was the skin glove from the left hind leg of the same frog, with the *outside* of the skin turned *outward;* this skin was filled with Ringer's solution approximately isotonic with frog's blood, and was then suspended at different temperatures in the same solution. The behavior shows that during the first six hours there was not a continuous transport of water through the skin and that even at the end of twenty-four hours' suspension the gain in weight of the glove was insignificant. Since in the control the normal direction in which water is transported through the integument is from without inward, we may infer that in the control the loss in weight observed during the second hour is due to a loss of water by the tissue

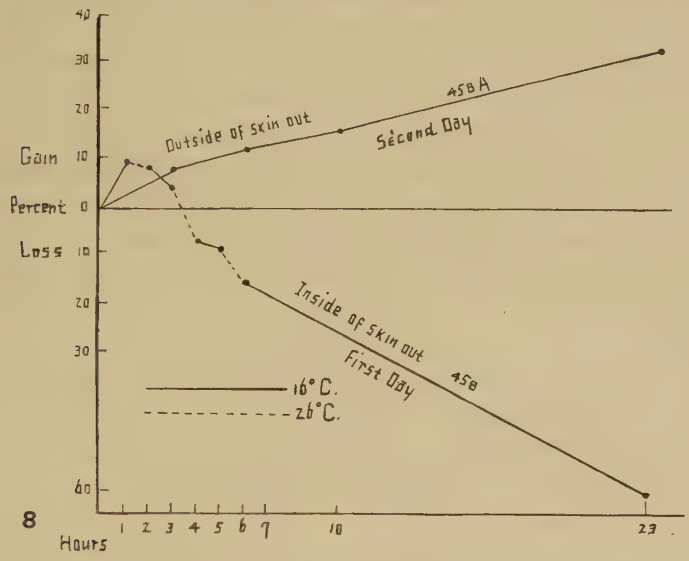
colloids rather than to osmotic pressure. The same is observed in other cases (fig. 4) where the outside of the skin is turned outward.

Although the behavior just mentioned (exper. 457 A, fig. 7) has frequently been observed, it is by no means uniformly met with. As in the case of skin gloves with the outside of the skin turned outward (fig. 2), cases are found in which fluid is transported through the skin in a continuous manner when gloves filled with Ringer's solution approximately isotonic with frog's blood, with the *inside* of the skin turned *outward,* are alternately suspended in the same solution at different temperatures. This behavior is shown in the following experiment.

Experiment 458 (fig. 8) was made on a skin glove removed from the right hind leg of a frog (R. pipiens). The glove, with the *inside* of the glove turned *outward,* was filled with 6 cc. of Ringer's solution isotonic with frog's blood, and then was alternately suspended for definite periods of time in this same solution at temperatures of 16°C. and 26°C. After the usual initial gain in weight obtained during the first hour's suspension, the glove lost weight continuously at both low and high temperatures, and in this respect its behavior differed from that observed in experiment 457 A (fig. 7). The loss in weight of the glove was continuous; but it will be noted that during certain hourly periods the loss was greater at 26°C. than at 16°C. This may be accounted for as follows: at 16°C. and at 26°C. water was transported from the glove through the skin outward. At 16°C., however, the skin itself absorbed, and at 26°C. it lost water. In this way it may be possible to explain the greater loss at the higher temperature.

After twenty-three hours' suspension in Ringer with the inside of the skin turned outward, the glove was removed from the glass cylinder, and both surfaces of the skin were carefully dried between sheets of filter-paper. The same glove was then again fastened to a glass tube with the *outside* of the skin turned *outward,* instead of *inward,* was filled with 6 cc. of Ringer's solution isotonic with frog's blood, and was

suspended in the same solution at 16°C. The behavior of the glove (458 A), with the outside of the skin turned outward, was typical of that observed in most experiments of this character. It gained weight continuously from the time it was first suspended in Ringer's solution (fig. 8, second day). A comparison of the behavior observed on the first and on the second days clearly still further emphasizes the fact that water is more readily absorbed by the skin through its inner surface than through its outer, and that the direction in which



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water is transported through the skin in opposition to osmotic pressure depends upon which surface of the glove is turned inward or outward.

As all of the experiments mentioned in the preceding pages were made on skins removed from living frogs, we may infer that the behavior observed, at least during the first few hours of the experiment, represents the average condition one would find in the power of the living skin both to absorb water and to transport water through itself in opposition to osmotic pressure. It has been observed in the majority of instances that water is transported through the skin in a

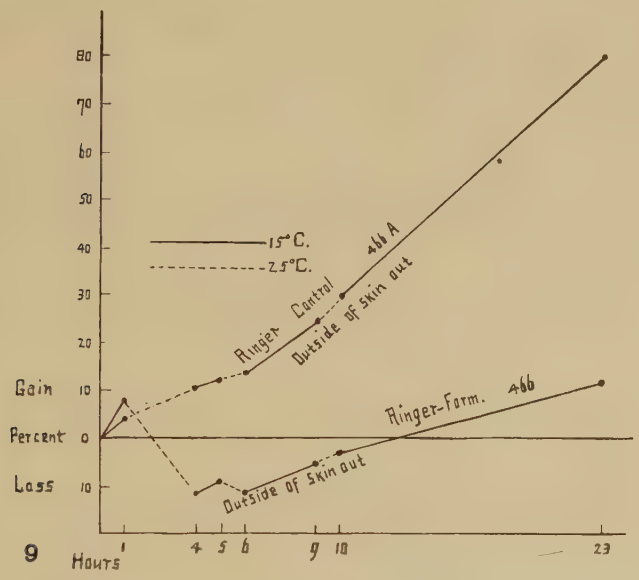
definite direction and in a continuous flow when skin gloves filled with Ringer's solution approximately isotonic with frog's blood are suspended in the same solution either at a constant temperature or at different temperatures. Also, when fluctuations in the weight of the glove occur, in the sense that the latter loses or gains weight in relation to differences in temperature, we have seen that the fluctuations appear to occur more readily in skins whose power to transport water through them in opposition to osmotic pressure either is lost or has become greatly diminished. If, therefore, by means of experiment we can eliminate the continuous transport of water through the skin in opposition to osmotic pressure, and if a gain or a loss of water by the glove then occurs, this must be due to the absorption or to the loss of water by the skin itself. When formaldehyde is added both to the Ringer's solution placed in the glove and also to the solution in which the glove is suspended, it has been found that the power of the skin to transport water through itself in opposition to osmotic pressure either is completely lost or is greatly diminished.

Two skin gloves removed from the right and the left hind legs of the same individual (R. pipiens) were used in the following experiments (experiments 466 and 466 A, fig. 9). The right skin glove, the control (exper. 466 A), with the *outside* of the skin turned *outward,* was filled with Ringer's solution approximately isotonic with frog's blood and was suspended alternately at 15°C. and 25°C. in the same solution. The behavior of this glove was typical of that observed in other similar experiments (fig. 2), where water was transported through the skin continuously.

The left skin glove (exper. 466, fig. 9) of the same frog, also with the *outside* of the skin turned *outward,* was filled with a 5 per cent solution of formaldehyde made up with Ringer's solution approximately isotonic with frog's blood, and was then suspended alternately at 15°C. and 25°C. in the same solution. In this case the behavior of the glove differed markedly from that of the control; the glove did not gain

weight continuously, but during the first six hours of its suspension gained weight at 15°C. and lost weight at 25°C. After the sixth hour, however, a continuous, though slight, gain in weight occurred up to the twenty-third hour of the experiment.

A corresponding set of experiments was made upon the right and the left skin gloves of another frog with the *inside* of the skin turned *outward*. The right glove, the control



(exper. 465 A, fig. 10); with the *inside* of the skin turned *outward,* was filled with Ringer's solution approximately isotonic with frog's blood, and was suspended in the same solution alternately at 15°C. and 25°C. The behavior of the control was similar to that previously described as commonly occurring in experiments of this character (fig. 8, first day). After the usual initial gain in weight, which occurred during the first hour of suspension, the glove lost weight continuously by a transport of water occurring through the skin outward.

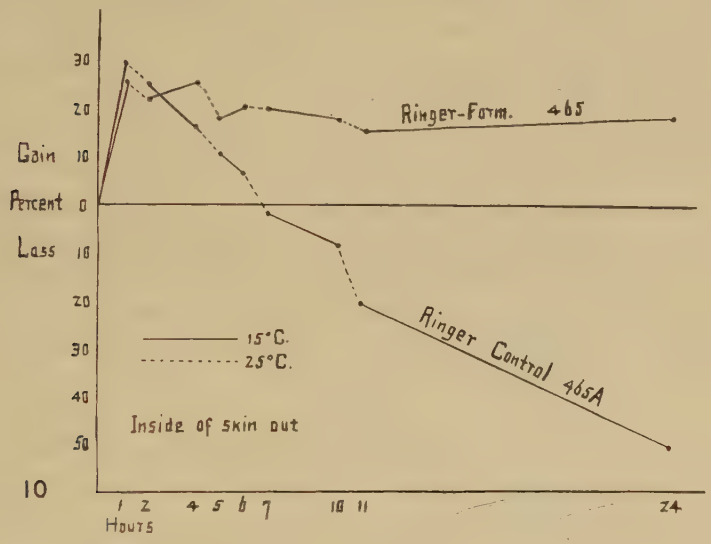
The left skin glove of the same frog (exper. 465, fig. 10), also with the *inside* of the skin turned *outward,* was filled with a 5 per cent solution of formaldehyde made up with Ringer's solution approximately isotonic with frog's blood, and was suspended in this same solution at 15°C. and 25°C. In this case, after the initial gain in weight which occurred during the first hour's suspension, the glove did not, like the control (465A), lose weight continuously when placed alternately in the solution at different temperatures; but between the second and the sixth hours of the experiment it lost weight at 25°C. and gained weight at 15°C.

While we are here undoubtedly dealing with dead skins, since the latter have been immersed in a formaldehyde solution, nevertheless the behavior of such skins emphasizes the probability that the corresponding fluctuations in weight observed in the living frog (fig. 5) and in skins removed from the body are for the most part due to the fact that the power of the skins to transport water through them continuously in opposition to osmotic pressure has been either greatly diminished or entirely destroyed. Such fluctuations in weight would therefore appear to be due to a gain or a loss of water by the skin itself. These fluctuations in weight of the skins are suggestive of the auxographic records made by MacDougal[7] on the succulent leaves of Mesenbryanthemum; these showed that the growing leaves of this plant lost water when exposed to high and gained water when exposed to low temperature.

Up to this point we have seen that the behavior of skin gloves, when filled with Ringer's solution approximately isotonic with frog's blood and suspended in the same solution, may be the resultant of the action or the interaction of several separate factors which may operate in harmony with or in opposition to one another. These factors are the power of the skin to absorb water, to release water, and to transport water through itself continuously in a direction in opposition

[7] MacDougal, D. T. 1920 Hydration and growth. Carnegie Institution of Washington.

to that of osmotic pressure. Should differences exist between the osmotic pressure of fluids which bathe the skin's opposite surfaces, the behavior of the skin glove would then be modified by osmotic pressure acting in conjunction with the above-mentioned factors. It is therefore desirable to analyze these several factors acting in conjunction with known differences in osmotic pressure.



V. THE BEHAVIOR OF GLOVES OF FROG'S SKIN, WITH THE *OUTSIDE* OF THE SKIN TURNED *OUTWARD,* WHEN FILLED WITH A HYPOTONIC RINGER'S SOLUTION AND SUSPENDED IN A HYPERTONIC RINGER'S SOLUTION APPROXIMATELY ISOTONIC WITH FROG'S BLOOD

In all the experiments thus far mentioned the Ringer's solution designated as being approximately isotonic with frog's blood consisted of 7 grams of NaCl, 0.3 gram of KCl, 0.25 gram of $CaCl_2$, and 0.03 gram of $NaHCO_3$ added to 1000 cc. of distilled H_2O. This is the formula given by Howell; in the following experiments it is referred to as the isotonic Ringer's solution. In the experiments about to be mentioned, in addition to this isotonic solution, there were used three other Ringer solutions which differ from the isotonic solu-

tion only in the amount of NaCl present. As compared with the isotonic solution, these three solutions are hypotonic and contain, respectively, 6.5, 6.25, and 6 grams of NaCl, added to 1000 cc. of H_2O instead of 7 grams, as in the isotonic solution.

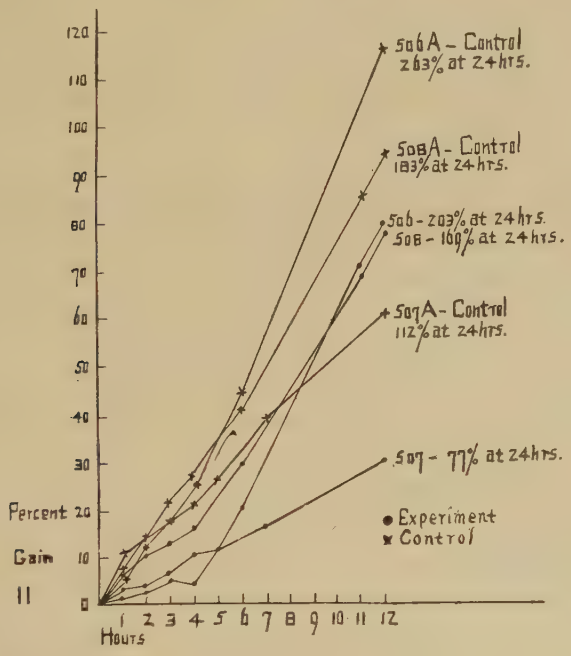
The following three sets of experiments (figs. 11, 12, and 13) illustrate the behavior of gloves of frog's skin, with the *outside* of the skin turned *outward,* when filled with a hypotonic Ringer's solution and suspended in a hypertonic Ringer's solution. The crosses (x) in the figures represent the behavior of the *controls;* that is, the behavior of the gloves filled with Ringer's solution approximately isotonic with frog's blood and suspended in the same solution. The circles represent the behavior of skin gloves filled with a *hypotonic* Ringer's solution and suspended in a *hypertonic* Ringer's solution which is approximately isotonic with frog's blood. In each case the gloves for the control and for the experiment were taken from the hind legs of the same frog.

1. The behavior of gloves of frog's skin filled with a Ringer's solution containing 6.5 grams of NaCl to 1000 cc. of H_2O, when suspended in a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O

Controls: The behavior of skin gloves filled with a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O, when suspended in the same solution.

The important feature illustrated by this set of experiments is that, as in the case of the controls, the skin gloves filled with hypotonic Ringer immediately gained in weight when placed in a hypertonic Ringer's solution. At the end of twelve hours' suspension in the solution, however, the amount gained by the gloves containing the hypotonic solution (experiments 506, 507, and 508, fig. 11) was less than that gained by the controls (experiments 506 A, 507 A, and 508 A, fig. 11), in which the gloves containing Ringer's solution approximately isotonic with frog's blood were suspended in the same solution. The fact that the rate at which water

was transported through the skin into the glove was less in the experiments than in the controls indicates that in the experiments some factor must have been acting in opposition to osmotic pressure by which, until an equilibrium had been established, water should have been driven outward through the skin from the glove. This factor, as we have previously demonstrated, is the force by which water is driven through the skin in a direction opposite to that of



osmotic pressure; and in the experiments this driving force exceeds that of the driving force of osmosis. We may thus account for the fact that skin gloves filled with a hypotonic Ringer's solution may gain weight when placed in a hypertonic Ringer's solution.

Another factor which may occasionally modify the behavior of skin gloves, under the conditions of the experiment above mentioned, is the capacity of the skin itself to gain or to lose water in conjunction with, but independently of, the

forces by which water is driven inward or outward through the integument.

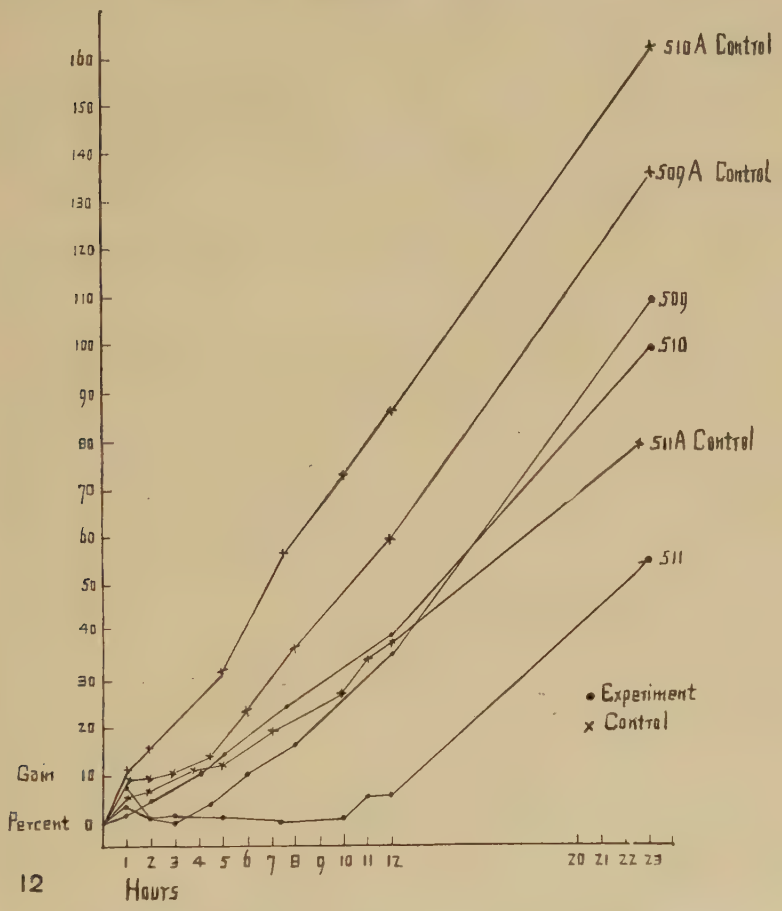
In experiment 506 (fig. 11) the temporary loss in weight of the glove between the third and the fourth hour is apparently due to a temporary loss of water by the skin itself.

2. The behavior of gloves of frog's skin filled with a Ringer's solution containing 6.25 grams of NaCl to 1000 cc. of H_2O, when suspended in a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O

Controls: The behavior of skin gloves filled with a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O, when suspended in the same solution

A still greater difference exists in the three following than in the last-mentioned experiments, between the osmotic pressure of the Ringer solution that is contained in the glove and that in which the glove is suspended. It will be observed that the controls of these three experiments (509 A, 510 A, and 511 A, fig. 12) gained in weight continuously from the time the gloves were first suspended in Ringer, and that the amount gained at the end of twenty-three hours' suspension was greatest in the control 510 A and least in 511 A. In experiment 510, of which 510 A is the control, the glove containing hypotonic Ringer gained weight continuously for a period of twenty-three hours and, except that the amount gained was less, its behavior was similar to that of its control. The circumstance that the gain in weight of this glove (510) containing hypotonic Ringer was continuous throughout the experiment might be explained by the fact that this particular skin may have possessed marked capacity to transport water through itself in a direction opposite to the force of osmotic pressure and at a rate which at all times exceeded that at which water was driven by osmosis through the skin outward. On the other hand, there is a marked difference between the behavior of the two remaining gloves and that observed in experiment 510 and in the three controls. In both cases in which the glove containing hypotonic Ringer

was suspended in hypertonic Ringer, the glove gained in weight during the first hour of its suspension (experiments 509 and 511). This gain in weight, however, was succeeded by a loss, which was followed by a gain that continued



through the remaining period of the experiment. It is evident that the initial and temporary gain in weight observed in experiments 509 and 511 could not be accounted for on the grounds of differences in osmotic pressure, but was more probably due to the presence of water absorbed by the skin itself. This temporary gain followed by a temporary loss in

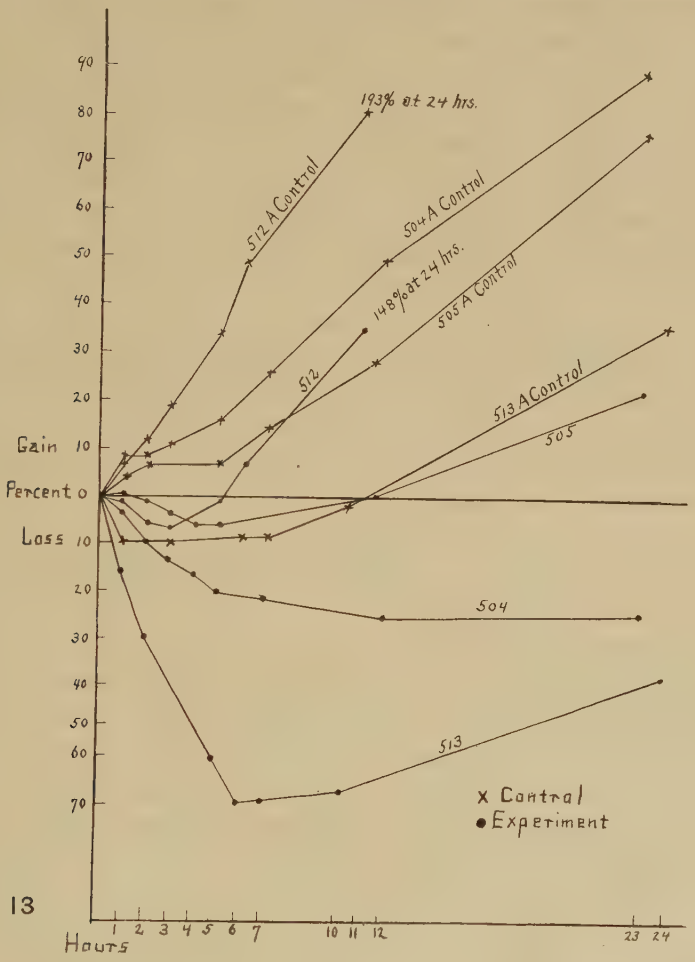
weight suggests that, after the hydration capacity of the skin had been satisfied, there was set up an osmotic transfer which continued either until an equilibrium had been established or until the driving force of osmosis was exceeded by the force which drives water through the skin in the opposite direction to osmotic pressure.

3. The behavior of gloves of frog's skin filled with a Ringer's solution containing 6 grams of NaCl to 1000 cc. of H_2O, when suspended in a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O

Controls: The behavior of skin gloves filled with a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O, when suspended in the same solution.

This set of experiments clearly illustrates the effects of the driving force of osmosis when this force is sufficiently great to overcome that force by which water is driven through the skin into the glove in opposition to osmotic pressure. In each of the four experiments graphically represented in figure 13, the skin glove containing a hypotonic Ringer's solution at first lost weight when suspended in hypertonic Ringer (experiments 504, 505, 512, and 513). In three instances, however, after an equilibrium had been established, the gloves began to gain weight and continued to do so until the termination of the experiment (experiments 505, 512, and 513). In this latter respect their behavior resembled that of their controls (505 A, 512 A, and 513 A) which, from the beginning of their suspension, gained weight continuously under conditions in which both surfaces of the skin were bathed by solutions having the same osmotic pressure. In the control 513 A the glove lost weight during the first hour of its suspension in Ringer; its weight then remained practically stationary for about six hours, after which time, however, there came a gain in weight. This form of behavior has already been demonstrated for certain skins in which the capacity to transport water through the skin in a direction opposite to that of osmotic pressure has been either greatly

diminished or entirely lost (exper. 442 A, fig. 3). The initial loss of water by the glove in control 513 A can be ascribed not to differences in osmotic pressure of the fluids which bathe the skin's opposite surfaces, but rather to a loss by



the skin itself of water which it had previously held. Also, it may be observed that in experiment 513 the initial loss in the weight of the glove greatly exceeds that observed in the gloves in the three other experiments (experiments 504, 505, and 512) as well as that in its control (513 A). It is evident

that we may account for the greater loss in weight of the glove in experiment 513 on the ground that in this particular skin, as in that of its control (513 A), there was acting in opposition to osmotic pressure no force by which water could be simultaneously driven through the skin into the glove.

It would appear that the behavior manifested by skin gloves, with the outside of the skin turned outward, when filled with a hypotonic Ringer's solution and suspended in a hypertonic Ringer's solution, may be the resultant of the action and interaction of the following separate and distinct factors: osmotic pressure; the capacity of the skin itself to gain and to lose water; and a force which drives water through the skin in a direction opposite to that of osmotic pressure. Also, we find that skin gloves filled with a hypotonic solution and suspended in a hypertonic solution begin to gain weight after an equilibrium has been established, in proportion to the capacity of the skin to transport water through itself when both its surfaces are bathed by solutions having the same osmotic pressure.

VI. ON THE TRANSPORT OF WATER THROUGH THE SKIN IN THE LIVING FROG WITH URETERS LIGATED, WHEN SUSPENDED IN A HYPERTONIC SALINE SOLUTION

It has been shown by the writer in a former paper ('19) that when the ureters have been ligated and a frog or toad is placed in water, there immediately follows a gain in body weight which may gradually result in an intense form of generalized oedema. These experiments should not be confused with those of Overton ('04), who ligated the cloacal opening, but not the ureters. When only the cloacal opening is ligated, the flow of urine through the ureters into the bladder and cloaca is not interrupted. In such cases, as stated by Overton, the bladder becomes filled with urine which backs up into the digestive canal. After the cloacal ligature is removed, this urine is readily expressed from the bladder and intestinal canal. On the other hand, after the ligature has been removed from the ureters, ordinarily no fluid can immediately be expressed from the cloaca, as in this case the

excess of fluid contained in the body has been deposited as lymph in the tissues and cavities of the body, and not as urine in the cloaca and intestinal canal. Therefore, only by ligating the ureters can a typical oedematous condition of the body be produced.

It has previously been observed by a number of writers that living frogs, as well as toads, lose weight when suspended in hypertonic saline solutions. We are now in a position to compare the behavior of a living frog whose ureters have been ligated with that of a skin glove filled with Ringer's solution isotonic with frog's blood, when each is suspended in a hypertonic solution. In the case of the living frog, with ureters ligated, the conditions are essentially analogous to those in the skin glove; each consists of a closed sac of frog's skin. In the experiment about to be mentioned and in others similar, the cloacal opening was ligated in addition to the ureters. This was done to insure against the fluid's escaping from the body in case an extravasation should occur at the point where the ureters had been ligated. The ligation was accomplished by passing under the ureters a silk ligature which was tied over the urostyle. The cloacal opening also was tied with a silk ligature. It is no easy matter in all cases to get what one may regard as a satisfactory ligation; injury to the skin by the needle is very likely to permit lymph in the dorsal subcutaneous lymph sinuses to extravasate through the openings. Unless the ligation is practically perfect, the frog or toad should not be used for experiments of this character. Better results have usually been obtained on frog's recently caught than on those which have remained for some time in the laboratory. This seems to be due to the fact that in pale-skinned winter frogs the skin is much thinner and is more easily injured than in those recently captured. On account of the toughness of their skin, toads have generally been found to be more favorable subjects for these experiments than are frogs.

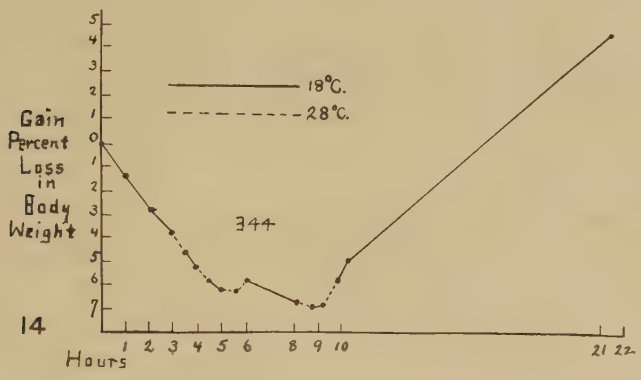
In the following experiment (344, fig. 14) a living frog, with its ureters and cloacal opening ligated, was fastened to

a frog board and was alternately suspended up to the armpits in a 0.9 per cent solution of NaCl at 18°C. and 28°C. The frog (R. pipiens) was obtained from a dealer on September 25th. The experiment, beginning on September 26th, extended over a period of about twenty-one hours, during which time the frog was suspended in a hypertonic saline solution. When a ligated frog is suspended in a solution in this manner, it is evident that no fluid can possibly enter or leave the body except by passing through the integument. When frogs are placed in finger-bowls containing a saline solution, fluid is often taken into the intestinal canal through the oral cavity; hence the method of suspending them on frog boards is essential to this type of experiment.

It will be observed that during the first nine hours of its suspension in the hypertonic solution (fig. 14), the ligated frog lost weight continuously, except during the sixth hour, when there was a slight gain. With the tenth hour, however, the frog began to gain in weight, and continued to do so until the close of the experiment. It is therefore seen that the behavior of the living frog with ureters ligated when suspended in a hypertonic saline solution (fig. 14) is identical with that of a skin glove, with the outside of the skin turned outward, when filled with Ringer's solution and suspended in a hypertonic Ringer's solution (fig. 13). It is evident that the loss in weight which immediately follows the suspension of the living frog in the hypertonic solution is, as in the case of the skin glove, due chiefly to differences in the osmotic pressure of the fluids which bathe the skin's opposite surfaces. Also, in each instance, after an equilibrium has been established and water is no longer driven by osmosis through the skin outward, a gain in weight of the frog and of the skin glove follows, due to the existence of a force which drives water inward through the skin in a direction opposite to that of the driving force of osmosis.

We know that when the living frog remains in water, water is driven by osmosis through the skin into the subcutaneous lymph sinuses, and that the amount of water supplied in this

manner to the body is influenced by temperature. In view of the observations made in the preceding pages, we have reason to believe that in the living frog, in addition to, though in conjunction with this osmotic transfer, water is also driven through the skin into the subcutaneous lymph sinuses by a force acting independently of osmotic pressure, so that the water supply to the body is not obtained exclusively



from that driven through the skin by osmosis. Furthermore, during the transport of water through the skin by either method, the skin itself may absorb or it may lose water. Should we therefore attempt to obtain the temperature coefficient of absorption of the skin, either in a skin glove or in the living animal, we should expect this coefficient to vary in relation to the capacity of any particular skin to transfer water through itself independently of the osmotic transfer, as well as to absorb or to lose water. A coefficient of absorption of the living skin should therefore be the resultant of the action and interaction of a number of factors which include osmotic pressure, a force which drives water through the skin independently of osmotic pressure, and a gain or loss of water by the tissue colloids.

VII. ON THE NATURE OF THE FORCE WHICH MAY DRIVE WATER THROUGH THE SKIN IN A DIRECTION COUNTER TO THAT CAUSED BY OSMOTIC PRESSURE

We have shown in the preceding pages that a number of factors may be concerned in the transport of water through the skin in the living frog, as well as through the skin when detached from the body. These factors include osmotic pressure, the capacity of the skin itself to absorb and to lose water, and a force which acts simultaneously and in conjunction with osmotic pressure, though independently of this. Having demonstrated the rôle played by each of these factors when acting either independently or in conjunction with one another, we must now analyze more closely the nature of the force which is able to drive water through the skin in a direction opposite to that of osmotic pressure.

In 1918, Gunzberg[8] published a paper dealing with the pathology of oedema, in which he advanced the view that the older theories of filtration, osmosis, and the affinity of colloids for water, do not afford an adequate explanation of oedema, and that the passage of water into the intercellular spaces is due to the electrical properties of the membrane separating circulating fluids from the tissues. He stated that the E. M. F. is due to membrane polarization; that the element K and the ions H and OH play an important rôle in this electrical osmosis, which is able to drive the fluid in a direction opposite to that of osmotic pressure. Gunzberg's view was based on experiments made on pithed frogs which were perfused with Ringer's solution and modifications of this solution; in these experiments the fluid entered by a canula in the aorta and left by the proximal end of this vessel. Gunzberg found that careful perfusion with a 0.7 per cent NaCl or with Ringer's solution caused no oedema. When, however, the Ringer's solution was deprived of K, there resulted a characteristic oedema in which the final weight showed as much as a 40 per cent increase in the

[8] Gunzberg, I. 1918 Contribution a l'étude de pathogenie de l'oedeme. Archiv. neer. de Physiol., vol. 2.

course of twenty-four hours. In such cases the lymph spaces and the pleuro-abdominal cavity were filled with fluid, and the stomach, bladder, and intestines produced an abundant secretion. Gunzberg found also that skin gloves filled with Ringer and immersed in a liquid with an excess or absence of K, increased in weight, while the weight of a glove filled with Ringer minus K and immersed in Ringer diminished.

In the light of the experiments described in the preceding pages, the writer has made a detailed study of the results obtained by Gunzberg. The results obtained by him would seem to indicate that the force by which fluid is transported through the skin, independently of osmotic pressure, may be regarded in the light of an electrical phenomenon.

A number of experiments were made on skin gloves, with the outside of the skin turned outward, as well as inward, to determine the rate of transport of fluid through the skin, in relation to the presence or absence of K both in the Ringer's solution contained in the glove and also in the solution in which the glove was suspended. Skin gloves removed from the hind legs were used; the glove from the right leg was, as a rule, used for the control and the glove from the left leg of the same frog was used for the experiment. Also, as in all of the preceding experiments, the gain or the loss in weight of the glove was based on the original weight of the empty skin glove, before fluid was placed in the glove, and prior to the suspension of the glove in the solution. The results of these experiments are graphically shown in figures 15 to 23, inclusive. The variability observed in the behavior of different skins in the same type of experiment, in relation to the rate at which fluid is transported through the skin independently of osmotic pressure, is analogous to that previously described as commonly existing among skins obtained from different animals (figs. 1 to 4, inclusive). A knowledge of this variability for skins in general has therefore made possible a correct interpretation of the variable conditions observed in these experiments.

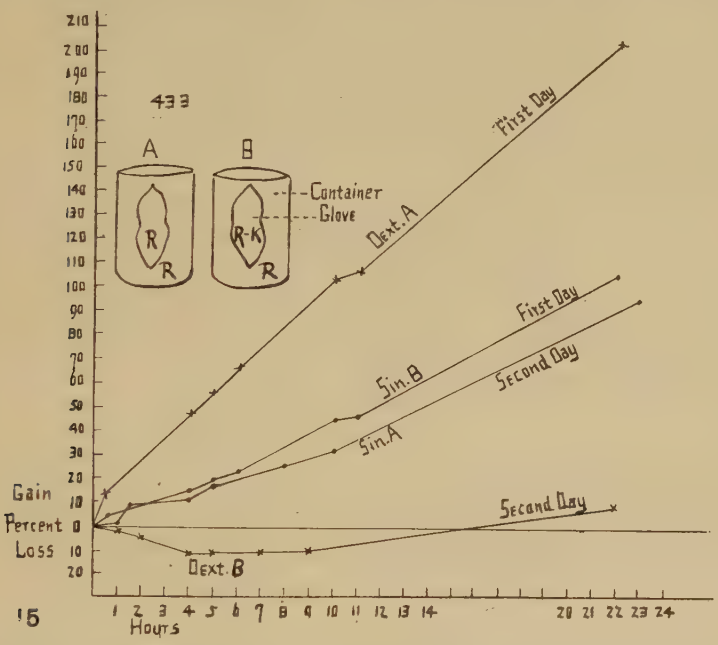
First, experiments were made to determine what rôle the element K generally plays in influencing the velocity of diffusion of water through frog skins taken from different animals of the same and of different species. After this had been determined, special type experiments were made on skin gloves, with the outside of the skin turned outward as well as inward, in which the conditions were modified in various ways, as regards the presence and absence of K in the Ringer's solution contained in the glove and in the solution in which the glove was suspended.

Experiment 433 (fig. 15)

The right and the left skin gloves of the same frog (R. pipiens), with the *outside* of the skin turned *outward,* were first filled, respectively, with Ringer's solution approximately isotonic with frog's blood and with Ringer minus K, and were then suspended in Ringer's solution. (Diagrams A and B in fig. 15 illustrate the type of the experiment, as regards the character of the Ringer's solution in the glove and that of the Ringer in the container in which the glove was suspended.) During the course of twenty-two hours' suspension, the right glove, which contained Ringer, and which in this instance served as the control (*Dext. A, first day*), gained more in weight than did the left glove which contained Ringer minus K (*Sin. B, first day,* fig. 15). At the end of twenty-two hours, the right glove was filled with Ringer minus K, instead of Ringer (*Dext. B, second day*), and the left glove with Ringer, instead of Ringer minus K (*Sin. A, second day,* fig. 15), and again both gloves were suspended in Ringer for another period of twenty-two hours. Figure 15 shows graphically that, during the second day's suspension, the left glove containing Ringer in which K was present (*Sin. A, second day*) gained more in weight than the right glove in which K was absent (*Dext. B, second day*). The behavior of the control on the first day (*Dext. A, first day*) signifies a marked capacity of the skin of this particular frog to transport water through itself under conditions in which both surfaces of the

skin are bathed by solutions having the same osmotic pressure.

One often finds skins through which, soon after their removal from the frog, fluid does not immediately diffuse when they are filled with Ringer's solution isotonic with frog's blood and are suspended in the same solution (fig. 3). In such cases it has been found that the presence or absence of K in the Ringer's solution contained in the glove does not



influence the behavior. After a more or less prolonged suspension of the glove in Ringer, however, a transport of fluid through the skin is usually initiated, and the weight of the glove containing Ringer in which K is present then increases at a greater rate than that of the glove containing Ringer in which K is absent. In other words, only in the case of skins in which a transfer of fluid is actually being accomplished is the behavior of the glove influenced by the presence or absence of K in the solutions. An example of this form of behavior is illustrated by the following experiment.

Experiment 429 (fig. 16)

The right and the left skin gloves of the same frog (R. pipiens), with the *outside* of the skin turned *outward,* were filled, respectively, with Ringer and Ringer minus K and were then suspended in Ringer's solution. (As in fig. 15, diagrams A and B indicate the type of experiment.) The behavior of the gloves showed that in neither case was there a continuous transfer of fluid through the skin during the first eight hours' suspension in Ringer. From the eighth hour on, however, water began to diffuse through the skin into the gloves, and a gradual gain in weight of the gloves followed. At the end of twenty-three hours' suspension, the weight of the glove containing Ringer in which K was present (A, fig. 16) was greater than that of the glove in which K was absent (B, fig. 16). Why, under the conditions of the experiment, water should not be transported through the skin at the time of its removal from the body, but should be later on, has not up to the present time been determined. Such is the case, however, and it is evident that the recognition of this fact explains the aberrant behavior of the gloves in question.

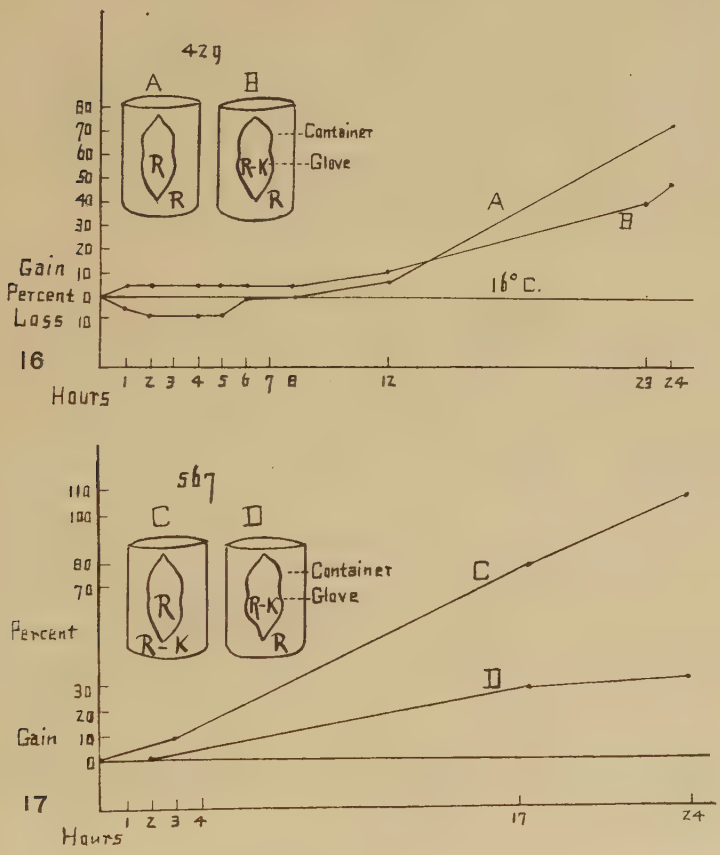
The writer made sixteen preliminary experiments of the type illustrated by figures 15 and 16, in all of which the results were essentially uniform, in the sense that they conformed with those of the experiments mentioned by showing that the ions of K influence the velocity of diffusion.

We may now proceed to the consideration of a series of experiments based upon those above mentioned, in which the conditions have been still further modified.

Experiment 567 (fig. 17)

Two skin gloves removed from the hind legs of the same frog (R. pipiens), with the *outside* of the skin turned *outward,* were filled, one with Ringer and the other with Ringer minus K, and were then suspended, respectively, in Ringer minus K and in Ringer. (See diagrams C and D in fig. 17 for type of experiment.) Four experiments of this character were made,

in three of which the behavior observed was essentially similar to that graphically shown in figure 17, in which it is seen that during the course of twenty-four hours the glove containing Ringer (C) gained more in weight than the glove containing Ringer in which K was absent (D). In the other



case, however, as in experiment 429 (fig. 16), a transfer of water did not take place in either skin until after the gloves had been for some time suspended in the solutions. After a transfer had been initiated, however, the glove containing Ringer, in which K was present, began to gain weight in excess of the glove containing Ringer in which K was absent.

It has previously been demonstrated (fig. 6) that, when the *inside* of the skin is turned *outward,* the direction in which fluid is transported through the skin is exactly opposite to that in which fluid is transported when the *outside* of the skin is turned *outward.* If the presence of K in the Ringer's solution influences the rate at which fluid is transported inward through the skin into the glove, when the outside of the skin is turned outward, it is evident that the rate at which fluid is transported outward through the skin from the glove when the inside of the skin is turned outward should be similarly influenced. Such has been found to be the case, as is shown by the following experiments which are of the same type as the preceding experiment (exper. 567, fig. 17).

Experiment 553 (fig. 18)

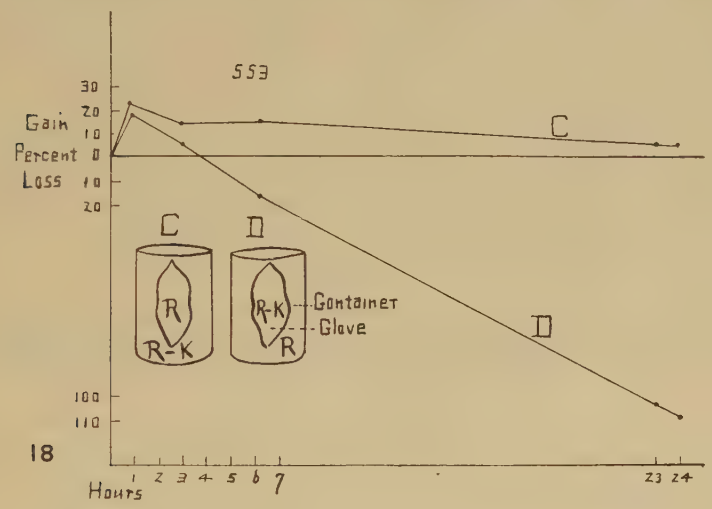
Two skin gloves removed from the hind legs of the same frog (R. pipiens), with the *inside* of the skin turned *outward,* were filled, one with Ringer and the other with Ringer minus K, and were then suspended, respectively, in Ringer minus K and Ringer. (For type of experiment see diagrams C and D in fig. 18.) Four experiments of this character were made, and in each instance, after the usual initial gain in weight during the first hour's suspension, the glove containing Ringer minus K (D in fig. 18), when suspended in Ringer, lost more in weight during the course of twenty-four hours than did the glove containing Ringer when suspended in Ringer minus K (C in fig. 18).

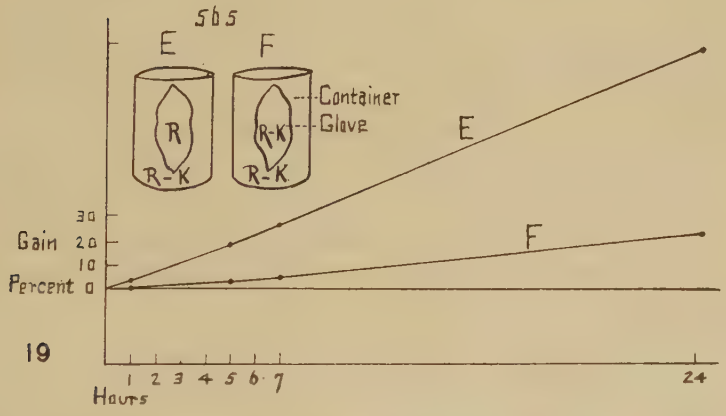
Another type of experiment which differs from that of the preceding experiments follows.

Experiment 565 (fig. 19)

The right and the left skin gloves of the same frog (R. pipiens), with the *outside* of the skin turned *outward,* were filled, one with Ringer and the other with Ringer minus K, and were then suspended in Ringer minus K. (See diagrams E and F in fig. 19, which indicate type of experiment.) Four experi-

ments of this character were made. In two instances the behavior of the gloves was similar to that graphically represented in figure 19, in which the glove containing Ringer (E, fig. 19) gained more in weight during the course of twenty-





four hours than did the glove containing Ringer in which K was absent (F, fig. 19). In the two other experiments the presence or absence of K in the solutions did not, during the first six hours of their suspension, influence the behavior of the gloves. Later on, however, the glove containing Ringer in which

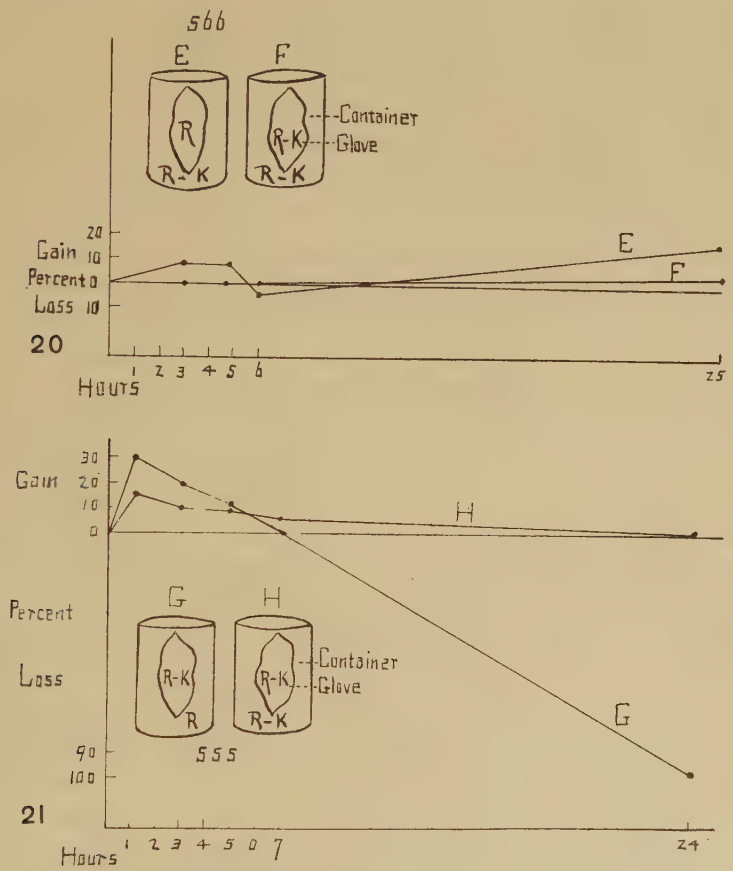
K was present began to gain weight in excess of the glove containing Ringer in which K was absent. This form of behavior, as previously shown (fig. 16), is characteristic of certain skins through which, at the time of their removal from the body, water does not immediately begin to diffuse when both sides of the skin are bathed by solutions having the same osmotic pressure. In one of these two experiments the initial gain followed by the loss in weight (E, fig. 20) observed during the first six hours of its suspension, is probably due to the absorption and the loss of water by the skin itself, and not to a transport of fluid through the skin into the glove during the first three hours, and a transport from the glove through the skin outward in the fifth and sixth hours.

Eight more experiments of still another type were made on skin gloves in which the *inside* of the skin was turned *outward*.

Experiment 555 (fig. 21)

The right and the left skin gloves of the same frog (R. pipiens), with the *inside* of the skin turned *outward*, were filled with Ringer minus K and were suspended, respectively, in Ringer and Ringer minus K. (For type of experiment see diagrams G and H in fig. 21.) In five of these experiments the behavior of the gloves was essentially similar to that graphically shown in figure 21, in which, during the course of twenty-four hours, the glove suspended in Ringer (G) lost more in weight than did the glove containing Ringer minus K when suspended in Ringer in which K was absent (H, fig. 21). In each instance a loss in weight of the glove was preceded by a gain, which occurred during the first hour's suspension. This gain in weight, we have seen, is probably due to an addition of water to the skin itself, through imbibition. In three of these experiments a transfer of water through the skin did not take place until after the gloves had been suspended for several hours in the solutions. After the transfer had been established, however, the rate of diffusion was found to be influenced by the presence of K in the solution in which the gloves were suspended. When we compare

the behavior observed in experiment 555 with that observed in the preceding experiments, it becomes evident that the presence of K accelerates the rate of diffusion of water through the skin in that particular direction through which, under conditions of the experiment, a diffusion is normally accomplished.



We have previously shown that the force which may drive water through the skin into the glove, when both surfaces of the skin are bathed by solutions having the same osmotic pressure, may also drive water through the skin when skin gloves containing Ringer are suspended in a hypertonic

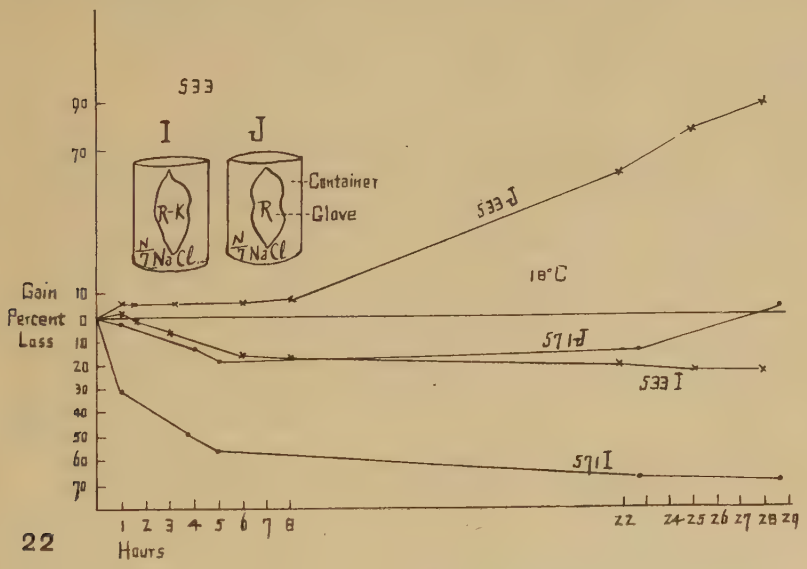
Ringer's solution (figs. 11, 12, and 13). In the following experiments we show how the action of this force may be modified by the presence or absence of K in the Ringer's solution.

Experiments 533 and 571 (fig. 22)

The right and the left skin gloves of the same frog (R. pipiens with the *outside* of the skin turned *outward,* were filled, respectively, with Ringer minus K and Ringer, both approximately isotonic with frog's blood, and were then suspended in a hypertonic saline solution (N/7 NaCl). (See diagrams I and J in fig. 22 for type of experiment.) Six experiments of this character were made; the general form of behavior manifested by the gloves in two of these experiments is graphically shown in figure 22.

In experiment 571 both gloves lost weight during the first five hours of their suspension in the hypertonic solution, and in the glove containing Ringer in which K was absent (571 I) the loss was greater than in the glove in which K was present (571 J). While, in each instance, this loss resulted from differences in osmotic pressure, the relatively slight loss in weight of one of the gloves (571 J) indicates that the force which drives water through the skin into the glove, in the opposite direction to that by which it is driven by osmosis, was greater in one skin than in the other. This increase in the rate of passage, due to the presence of K in the solution, counteracted the full driving force of osmosis, so that the glove containing Ringer in which K was present (571 J) lost less water and consequently less weight than did the glove containing Ringer in which K was absent (571 I) when the gloves were suspended in the hypertonic saline solution. From the fifth hour on, the glove containing Ringer in which K was present (571 J) gradually made a gain which amounted to about 92.5 per cent at the end of forty-six hours' suspension in the hypertonic solution. This gradual gain indicates that after an equilibrium had been established between the Ringer's solution in the glove and the NaCl solution in which

the glove was suspended, water was driven through the skin into the glove by some force other than osmotic pressure and that the velocity of the transfer was increased by the presence of K in the Ringer's solution. On the other hand, after the fifth hour of suspension the behavior of the glove containing Ringer in which K was absent (571 I) differed somewhat from that of the glove containing Ringer in which K was present (571 J). In this instance an equilibrium had been established sometime between the fifth and the twenty-second



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hour; the weight of the glove then remained stationary for a considerable time, after which, at the end of forty-eight hours' suspension in the hypertonic solution, there followed a slight gain in weight amounting to about 13 per cent.

In relation to the presence and absence of K in the Ringer's solution, the general behavior of the gloves in experiment 533 (fig. 22) is the same as in experiment 571 (fig. 22). The fact that the initial loss in weight of the glove in experiment 571 was greater than in experiment 533 may be explained on the ground that the capacity of the skin to transfer water through

itself in a direction opposite to that of osmotic pressure was less in experiment 571 than in experiment 533.

In the above experiments we have shown how the driving force of osmosis may be modified by the presence of K in the Ringer's solution contained in the glove when the latter, with the *outside* of the skin turned *outward*, is suspended in a N/7 NaCl solution. Let us now reverse the conditions and study the behavior of gloves with the *inside* of the skin turned *outward*, when filled with N/7 NaCl and suspended, respectively, in Ringer minus K and in Ringer. As in the other experiments, the Ringer's solution used was approximately isotonic with frog's blood.

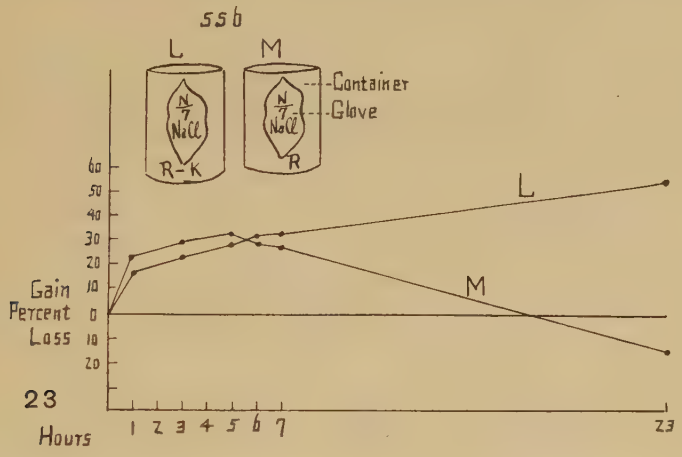
Experiment 556 (fig. 23)

Four experiments of this character were made and, in three instances, the behavior observed was essentially similar to that of the gloves graphically represented in figure 23. (For type of experiment see diagrams L and M in fig. 23.) The essential feature to be noted is that after an equilibrium had been established, the glove suspended in Ringer in which K was present (M) began to lose weight, on account of a transfer of water from the glove through the skin outward—that is, in the normal direction taken by the transfer when the *inside* of the skin is turned *outward*.

VIII. ON A POSSIBLE EXPLANATION OF THE CONTINUOUS FLOW OF LYMPH BETWEEN THE CAPILLARIES OF THE BLOOD-VASCULAR SYSTEM AND THOSE OF THE BLINDLY-ENDING LYMPHATICS

The results of the experiments detailed in the preceding pages lead us to the conclusion that when the living frog remains in water, three sets of factors may be involved in the transportation of fluid through the skin into the subcutaneous lymph sinuses. These factors constitute, 1) the varying hydration capacity of the tissue colloids by which the skin itself may absorb or lose water; 2) osmotic pressure, and, 3) a force, electrical osmosis, which acts independently of, and simultaneously with osmotic pressure, in driving water through the skin into the subcutaneous lymph sinuses.

After fluid has been transported through the skin into the subcutaneous lymph sinuses, it is then conveyed from the latter in the form of lymph to the blood-vascular system, where it mingles with the blood plasma. Some of this blood plasma is then transported in the form of lymph through the walls of the blood capillaries into the tissue spaces, from which it may be taken up and held by the tissue colloids and be collected by the blindly-ending lymphatics which return it to the blood-vascular system. In other words, when the living frog remains in water, a continuous flow of fluid is taking place through the skin into the subcutaneous lymph



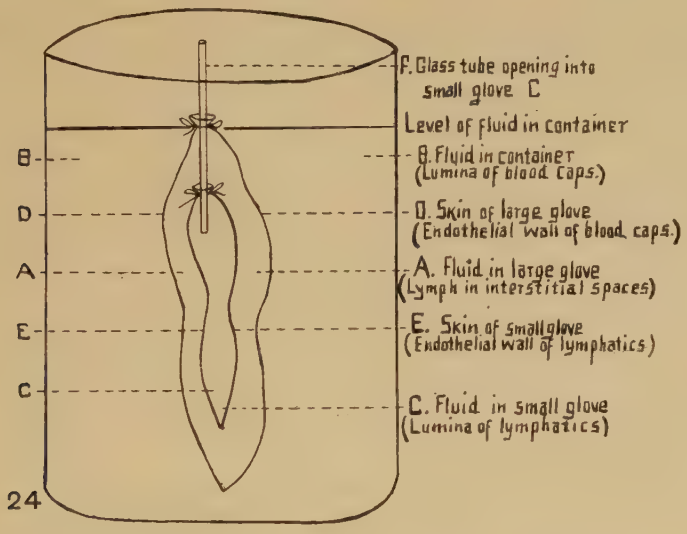
sinuses, and thence through the blood-vascular system and tissue spaces back again into the lymphatic system.

On the assumption that the same factors are involved in the transportation of fluid through the endothelial walls of the capillaries of the blood-vascular and lymphatic systems, as through the skin into the subcutaneous lymph sinuses, we have at least a working hypothesis to account for the establishment and maintenance of a continuous flow of fluid between the blood capillaries and those of the blindly-ending lymphatics. In any event, on this assumption, we can demonstrate a somewhat analogous condition by the following experiment which is based on the results of experiments mentioned in the preceding pages.

If a large glove of frog's skin (A, fig. 24), with the *outside* of the skin turned *outward,* is filled with Ringer's solution isotonic with frog's blood and is then suspended in a container (B) filled with the same solution, we have seen that fluid can be transported continuously from the container through the skin (D) into the glove (A). If a smaller glove (C, fig. 24), with the *outside* of the skin turned *outward,* is now filled with isotonic Ringer, and is placed inside the larger glove (A, fig. 24), water will be transported continuously from the large glove (A) through the skin into the smaller glove (C). Now, with both surfaces of both skin gloves bathed by solutions having the same osmotic pressure, we may expect to establish a continuous flow of fluid passing from the container (B) through both skins (D and E) into the smaller glove (C). Any excess of fluid in the smaller glove (C) would flow from the latter into the capillary tube (F).

If, in either of the two gloves, we should substitute for the Ringer's solution isotonic with frog's blood a Ringer solution having a slightly lower osmotic pressure, we know that water will continue to be transported through the skin in the same manner and direction. If a Ringer's solution containing 6.5 grams of NaCl to 1000 cc. of water, instead of 7 grams as in the so-called isotonic solution, were placed in the smaller glove (C), water would still continue to be transported through the skin from the large into the smaller glove, in the opposite direction to osmotic pressure. (See experiments 506, 507, and 508, fig. 11.) Also, if this same hypotonic Ringer solution were substituted for the isotonic solution in the larger glove (A), water in container B would still continue to be transported in a continuous manner through the skin into the larger glove and through the skin into the smaller one (C), even though the latter contained a Ringer solution of the same or of a slightly higher concentration than that in the larger glove. Also, it is evident that the rate at which fluid would be transported through the skin might be modified by the presence or absence of K in the solutions. (See experiments illustrated by figs. 15 to 23, inclusive.)

If we assume that B in figure 24 represents the lumina of the blood capillaries containing blood plasma; A, the interstitial spaces, containing lymph which has diffused through the walls of the blood capillaries (D); E, the walls of the blindly-ending lymphatics, and C, the lumina of the lymphatic vessels, containing lymph which has diffused through the endothelial walls of the lymphatics (E) from the interstitial spaces (A), we can picture how, under slightly varying con-


F. Glass tube opening into small glove C
Level of fluid in container
B
B. Fluid in container (Lumina of blood caps.)
D
D. Skin of large glove (Endothelial wall of blood caps.)
A
A. Fluid in large glove (Lymph in interstitial spaces)
E
E. Skin of small glove (Endothelial wall of lymphatics)
C
C. Fluid in small glove (Lumina of lymphatics)
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ditions of the electrolytes and of the concentration of the body fluids, a continuous flow of fluid might be established between the blood capillaries and the lymphatics. Also, as in the case of the frog's skin, it is quite possible that the normal physiological direction in which fluids are transported through the endothelial walls may depend upon which surface of the capillary wall is directed outward or inward, so that, if we could experimentally turn the inner surface of the lymphatic capillaries outward, lymph might then diffuse into the tissue spaces from the lymphatics.

We may therefore assume the possibility that the flow of fluid between the blood capillaries and the lymphatics may be

established and continued in the manner suggested. One important question still remains to be determined—whether the rate of passage through the skin, as observed in the experiments, is consistent with that which actually prevails in the living animal. Be this as it may, the significance of these observations cannot be disregarded in an attempt to account for the establishment and the continuance of a flow of fluids between the blood capillaries and the lymphatics, and for variations in the rate of flow, due to differences in potentiality which actually exist on opposite surfaces of the skin in the living animal.

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AN EXPERIMENTAL ANALYSIS OF OEDEMA IN THE FROG, WITH ESPECIAL REFERENCE TO THE OEDEMA IN RED-LEG DISEASE

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AN EXPERIMENTAL ANALYSIS OF OEDEMA IN THE FROG, WITH ESPECIAL REFERENCE TO THE OEDEMA IN RED-LEG DISEASE

The determination of the causation of oedema, which is associated with such a variety of pathological conditions, would appear to be a highly complex problem. Attempts have been made to show that there is but one primary cause which accounts for its presence whenever and however it manifests itself; on the other hand, there have not been a few investigators who have claimed that it is due, not to a single cause, but to a number of causes.

Oedema is defined as the excessive accumulation of lymph in the tissues and cavities of the body. When the excess of lymph is generally distributed throughout the body, we speak of a generalized oedema; when the excess is confined to a single locality or organ, the oedema is localized. The amount of lymph in the tissues of an animal may at different times vary considerably; it is only when the accumulation is clearly greater than normal that we have an oedematous, or pathological condition. To the attainment of this, water is essential. On account of the habits of the frog, and the free and continuous passage of water through its integument, this animal is a particularly favorable object for an experimental study of oedema.

Former investigations have given us, regarding the frog, many facts pertinent to the present study. We know that it is through osmosis (Maxwell, '13) that water is constantly and continuously driven through the integument into the subcutaneous lymph sinuses (Volhard, '12), and that at high temperatures it is driven through more rapidly than at low. We know also that when the normal frog remains in water at a temperature within the range of that of its natural

environment, a balance is soon established between the amount of water driven through the integument into the subcutaneous lymph sinuses and that excreted by the kidneys (Overton, '04), so that the relatively large amount of free water continuously added to the lymph does not accumulate in the body in excess. A normal frog[1] transferred from water at one temperature to water at another ordinarily either loses in weight or remains stationary; and in the cases where it gains weight, the gain is never so continuous through an extended period of time as to produce an oedema. It is therefore evident that when lymph continues to accumulate in the body in excess of normal, the gradual and continuous increase in body weight must be attributed to some factor or factors other than normal, which inhibit the establishment of the balance normally existing in the body between the flow of incoming and outgoing water. Whatever it may be in other animals, the explanation of oedema in the frog depends on the determination of such factor or factors.

In its passage through the capillary vessels of the blood-vascular system, some of the plasma escapes, or transudates, through the capillary walls into the tissue spaces, where it is either taken up by the tissues or returned to the lymphatics. The transudate constitutes what is ordinarily regarded as the lymph concerned in oedema; and all existing theories of oedema attempt to explain the process by which this lymph accumulates and is retained in excess of normal in the tissues and cavities of the body. An oedema of this character may be spoken of as an intracellular (interstitial?[2]) oedema, since, owing to the presence of the lymph in excess of normal in the tissue cells, tissue spaces, and cavities of the body, the oedema is evidenced chiefly by a swelling of the tissues in which the lymph has accumulated. Under certain pathological conditions, this form of oedema occurs in the frog,

[1] Unless otherwise stated, Rana pipiens (Screber) is the species of frog used in all the experiments of this investigation.

[2] It may be regarded as largely an academic question whether the oedema is purely intracellular or interstitial in character; while without doubt it is largely interstitial, it must to some extent also be intracellular.

as in all other vertebrates. In the frog, however, oedema manifests itself in yet another form.

When the water is driven by osmosis through the integument of the frog into the subcutaneous lymph sinuses, it there mixes with the lymph already present, received from the tissue spaces, and with the chyle received from the intestinal lymphatics. Through the lymph hearts the lymph thus formed finally reaches the blood-vascular system, where it mixes with the blood plasma. When for any reason more water enters the subcutaneous lymph sinuses than can be simultaneously excreted by the kidneys, free lymph will accumulate in excess of normal in these sinuses. In the frog the subcutaneous oedema is always accompanied by the intracellular (interstitial?) form, and vice versa. The problem before us is the determination of the factors which in each instance account for the gradual accumulation of lymph in the body—that is, for the oedematous condition.

Let us first consider those factors which might account for the subcutaneous oedema already described.

Experiments previously made by the writer in 1919 have shown that the generalized oedematous condition of the body observed in certain young frog larvae developed under experimental conditions, resulted from a deficiency in development of the kidneys (pronephroi), which, therefore, were unable, within a given time, to excrete as much water as simultaneously entered the body through the integument. The result was that lymph, in excess of normal, accumulated in the body. The investigations of Howland ('16), and especially those of Swingle ('19), bear out these results in a most striking manner, by showing that an oedematous condition of the body invariably results in young amphibian larvae after the kidneys (pronephroi) have been extirpated.

It has also been demonstrated by the writer ('19) that when the ureters of adult frogs and toads have been ligated and the animals remain in water, a general oedematous condition of the body gradually develops and, as shown in table 1, the rate at which lymph then accumulates in the body is

greater at a high than at a low temperature. In experiments of this character in which the oedema was pronounced, the subcutaneous lymph sinuses all over the body were greatly distended with lymph, and in many cases it was observed that the excess of lymph had filtered into the abdominal cavity (ascites).

A generalized subcutaneous oedema results when the ureters have been ligated and the frog remains in water; a localized oedema of exactly the same character is produced when the leg of a frog has been ligated. In such cases water is constantly and continuously being driven by osmosis through the skin of the leg into the subcutaneous lymph sinuses, as is the case with the skin in all regions of the body when the frog is submerged in water. In the case of the ligated leg, however, lymph gradually accumulates, in excess of normal, in the lymph sinuses distal to the ligature, solely for the reason that the local efferent drainage system of the ligated leg has been completely shut off from the general circulation, through which the oedematous fluid would otherwise reach the renal vessels and the excess of free water in the blood become available for excretion by the kidneys. In proof of the fact that this localized subcutaneous oedema of the leg results from a local mechanical block in the drainage system, one may remove the ligature from the leg and, after placing the frog in water, one will see that gradually the limb regains its normal proportions. Furthermore, it is possible simultaneously to produce a localized and generalized subcutaneous oedema in the same frog by ligating the leg as well as the ureters. After a localized swelling of the leg sinuses has developed, as well as a generalized swelling of the rest of the body, if the ligature is removed from the ureters and the frog is placed in water, the generalized swelling of the body will gradually subside and only the local swelling on the leg will persist. If the leg ligature is then removed, the localized subcutaneous oedema of the leg will gradually subside, for the excess of lymph in the leg now has free access to the general circulation, and the excess of free water

added to the lymph is thereby made available for excretion by the kidneys.

The chief significance of the above-mentioned experiments is that we have demonstrated by experiment that a subcutaneous oedema can be produced in a frog by a purely mechanical block in the drainage system, and that the location of the block determines the place and extent of the oedema.

The writer has found that a subcutaneous oedema is invariably accompanied by the intracellular (interstitial?)

TABLE 1

Showing gain (per cent) in body weight in same living frog or toad, in which the ureters have been ligated, when successively placed in tap-water at different temperatures

	12°C.	22°C.	32°C.
	Per cent	*Per cent*	*Per cent*
Toad (June)		5.33 (1 hour)	15.2 (1 hour)
Toad (July)		6.24 (1 hour)	11.5 (1 hour)
Toad (July)		4.8 (1 hour)	19.9 (1 hour)
Toad (July)		4.71 (2 hours)	31.7 (2 hours)
Frog (March)	3.2 (3 hours)	11.4 (3 hours)	
Frog (March)	1.1 (3 hours)	3.3 (3 hours)	25.0 (3 hours)
Frog (April)	2.9 (3 hours)	6.4 (3 hours)	22.0 (3 hours)
Frog (July)		1.7 (1 hour)	4.8 (1 hour)
Frog (July)		2.82 (1 hour)	6.3 (1 hour)
Frog (July)		1.8 (1 hour)	8.6 (1 hour)
Frog (July)		2.7 (1 hour)	6.32 (1 hour)

Percentages based on original weight of frogs and toads.

oedema, in which lymph accumulates, in excess of normal, in or among the cells of the tissues. After a frog, whose leg has been ligated, has remained for about twenty-four hours in water, the gastrocnemius muscle of the ligated leg may be greatly swollen and, when removed from the body, is found to weigh considerably more than that of the leg which has not been ligated. The abnormal increase in size and weight of the muscle from the ligated leg, over that of the muscle from the leg not ligated, is undoubtedly due to the presence of lymph in excess of normal, which, in some manner, has

been taken up and held by the muscle tissue. An explanation of the process by which this has come about constitutes, in the frog at least, the problem of intracellular (interstitial?) oedema. Two leading theories have thus far been advanced in explanation of the causation of this form of oedema. These are the osmotic pressure theory, as advanced by J. Loeb ('98), and the colloid-chemical theory of Martin Fischer ('15).

We may state at the beginning that an experimental analysis of the conditions found in the frog decidedly indicates that the production of the intracellular (interstitial?) form of oedema is owing to differences which can be demonstrated to exist between the osmotic pressure of the oedematous lymph in the subcutaneous lymph sinuses and hence in the blood plasma, and that of the lymph in normal muscle tissue.

Let us first consider different experiments made upon the same living frog placed in water after the osmotic pressure of its body fluids has been raised beyond a point we may ordinarily regard as normal. We know that when a normal frog remains out of water for a considerable time, or is placed in a hypertonic saline solution, a loss in body weight follows owing to a loss of water from the body. When such a desiccated frog is placed in water, immediately it begins to gain in weight, and continues to do so until water has been driven by osmosis through the integument into the subcutaneous lymph sinuses sufficient in amount to restore the osmotic pressure of the body fluids to a point that may be regarded as normal. When this condition has been established, the normal frog either begins to lose in weight or else for a considerable time remains stationary; from this time on, however, it never gains weight continuously. Three experiments of this character—experiments in which the same frog, after desiccation, was successively placed in water at 17°, 23°, and 32°C.—are illustrated by curves *1*, *2*, and *3* in figure 1. The broken lines in the figure indicate the loss in body weight which resulted during the period the frog remained out of water; and the continuous lines, the subsequent behavior of the frog when placed in water. Such curves, denoting the

behavior of the frog, may be spoken of as desiccation recovery curves (*D.R.C.* in figure), and represent in a typical manner the general behavior of normal frogs observed under similar experimental conditions.

The three desiccation recovery curves (*1, 2,* and *3* in fig. 1) show that, immediately following the suspension of the desiccated frog in water, it gains weight continuously for a period varying between two and four hours. This signifies that during this time more water enters the subcutaneous lymph sinuses through the integument than is simultaneously excreted by the kidneys. On the other hand, when the body fluids have regained their normal osmotic pressure, the loss in body weight which follows (curve *1,* fig. 1) signifies that more water is then being excreted by the kidneys than simultaneously enters the subcutaneous lymph sinuses—a circumstance which may possibly be explained on the grounds that free water has been released from the tissue colloids and has become available for excretion by the kidneys. As shown by the desiccation recovery curve *2* in figure 1, the amount of water driven through the integument into the subcutaneous lymph sinuses may, in course of time, be equibalanced by that excreted by the kidneys. At such a time, slight fluctuations in body weight can be temporarily produced by raising or lowering the temperature of the water. As thus far observed by the writer, frogs usually gain slightly in weight when transferred from water at a high to water at a lower temperature, and lose in weight when transferred from water at a low to water at a higher temperature. Such fluctuations in body weight would appear to be due to a temporary gain or loss of water by the tissue colloids rather than to fluctuations in kidney function, since it can be demonstrated that in the normal frog the kidneys serve as passive agents, and are more than capable of excreting any excess of free water in the body made available to them, when the frog is in water at any temperature within the range of that of its natural environment.

Having considered the osmotic reactions which result when a normal desiccated frog is placed in water, we may now compare these reactions with those obtained when the same frog has again been desiccated and, before being put into water, has had injected into its subcutaneous lymph sinuses a hypertonic saline solution.

After injecting a hypertonic saline solution into the subcutaneous lymph sinuses of a normal desiccated frog and then placing the frog in water, we should expect to obtain a desiccation recovery curve of the same general character as that obtained on a desiccated frog that had not been injected. The only difference we should expect to find would be that in the case of the injected frog the curve would have a greater maximum height, owing to an increase in the osmotic pressure of the body fluids beyond that resulting from desiccation. Such a curve (*4*) is shown in figure 1, which illustrates the behavior of this frog, which had been desiccated by remaining twelve hours out of water, and into the subcutaneous lymph sinuses of which, before it was placed in water, 1 cc. of a 2.97 per cent solution of NaCl had been injected. The maximum height obtained by this NaCl curve (*4*, fig. 1) is observed to be much greater than that of any one of the three control curves (*1*, *2*, and *3*, fig. 1) obtained when, before being placed in water, the desiccated frog had not been injected with a hypertonic solution.

This increase in height of the NaCl curve (*4*, fig. 1) over that of the controls (*1*, *2*, and *3*, fig. 1), in which the desiccated frog had not been injected with the hypertonic saline solution, indicates that an increased amount of water is driven through the integument by the increased osmotic pressure of the lymph in the subcutaneous lymph sinuses of the injected frog. On this same frog was made another experiment differing from the former only by the fact that the frog was not desiccated before 1 cc. of a 2.97 per cent NaCl solution was injected, and the frog put into water. In this experiment the behavior of the frog is illustrated by curve 5 in figure 1, which shows that for the body fluids to regain their average normal osmotic

pressure less water was required than when the frog had been desiccated prior to the injection.

The above experiments show the general osmotic reactions which take place in a living frog placed in water after the concentration of salts in the body and, consequently, the

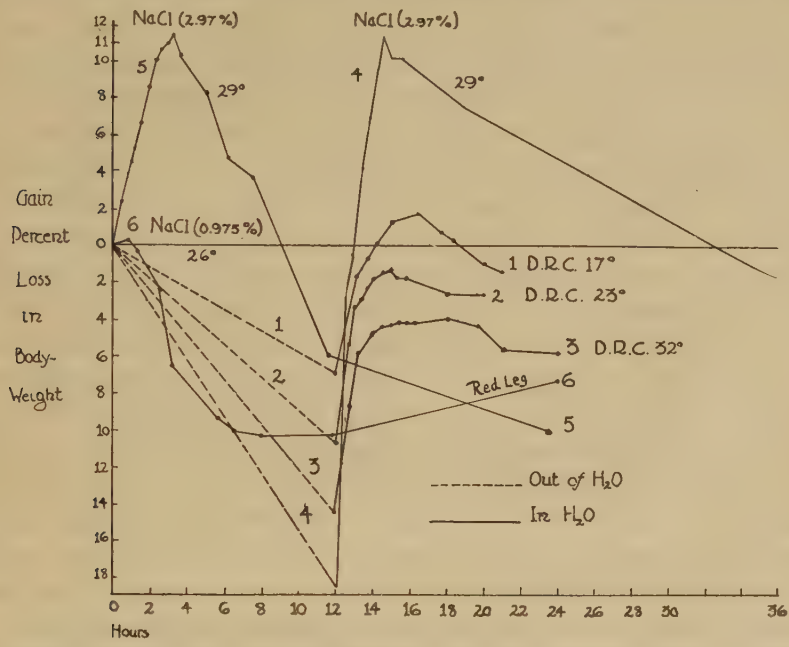

Fig. 1 Behavior of a normal desiccated frog when placed in water at different temperatures (curves *1*, *2*, and *3*); behavior of same frog when desiccated, after a saline solution has been injected into the subcutaneous lymph sinuses and the frog placed in water (curve *4*); behavior of same frog, not desiccated, after a saline solution has been injected into the subcutaneous lymph sinuses and the frog placed in water (curve *5*); behavior of same frog which has contracted red-leg disease during the course of the experiment (curve *6*).

osmotic pressure of the body fluids have been raised above normal. Let us now consider the behavior of muscles taken from desiccated frogs when these muscles are immersed in a Ringer's solution approximately isotonic with muscles of a normal, healthy frog that has not been desiccated.

It has long been recognized that the weight of a resting muscle taken from a normal healthy frog remains practically

stationary when placed in an isotonic saline solution, and that the same muscle would gain weight if placed in a hypotonic, and lose weight if placed in a hypertonic, saline solution. A solution of NaCl isotonic with frog's muscle has been estimated to vary between 0.6 (Nasse, '69) and 0.8 per cent at room temperature (Cooke, '98). Overton ('02) observed it to be 0.65 to 0.725 per cent, and Fletcher ('04) found 0.75 per cent to be the approximate value for the gastrocnemius at 17°C., when no fibrillary twitchings occur. It is thus evident that slight variations exist in the osmotic pressure of normal resting muscles taken from frogs of the same or of different species. A possible explanation of these slight variations, said by observers to exist in the osmotic pressure of normal resting muscles taken from frogs of the same or different species, may be due to the fact that they did not follow a uniform procedure prior to removing the muscles from the frogs. We know that when a frog remains out of water its body loses water; if this is not immediately replaced through the ordinary channels, there results an increase in the osmotic pressure of the body fluids. Experiments of the writer have shown that, in determining the osmotic pressure of frog's muscle, he can obtain uniform results only from the muscles of frogs which he has left in water a sufficient length of time to insure that through the agency of the kidneys a normal balance has been established between the incoming and outgoing flow of water. Only in such frogs could he insure against some degree of desiccation and be certain that the osmotic pressure of the body fluids was average and normal. It is therefore evident that muscles taken from frogs which are of the same species, but which may have come from different environmental conditions, will almost certainly show slight variations in osmotic pressure.

The $\triangle$ or freezing-point depression of a Ringer's solution approximately isotonic with normal resting gastrocnemius muscle taken from a frog which has remained in water at room temperature for at least twelve hours prior to the

removal of the muscle has been found to be approximately 0.445°. In the following pages such a solution will be spoken of as an isotonic Ringer's solution.

The following experiments show the behavior of muscles of desiccated frogs, when these muscles are immersed in the isotonic Ringer's solution above mentioned. In these experiments and all similar ones the writer removed the muscles from the body without injury to the tissues and followed a uniform procedure in drying the muscles with filter-paper before placing them on the balance. The experimental error involved in weighing the gastrocnemius muscle was found to be about 5 milligrams. Since in all of the experiments about to be mentioned the purpose was primarily to observe whether the muscles, when immersed in lymph or in the isotonic Ringer's solution, lost or gained weight continuously through a considerable period of time, weighings carried through two decimal places gave the facts with sufficient accuracy.

Two healthy normal frogs, originally weighing, respectively, 18.38 (A) and 28.7 grams (B), were kept out of water until A showed a loss in body weight amounting to 30 per cent and B a loss in body weight of 32.2 per cent. The four gastrocnemius muscles of these frogs were then carefully removed from the body without injury to the tissues, and each was placed at room temperature in a separate covered dish containing 20 cc. of the isotonic Ringer's solution. The behavior of each desiccated muscle of frogs A and B is shown in table 2, and can be compared with that of two gastrocnemius muscles similarly taken from a normal healthy frog (C) not desiccated, and immersed in 20 cc. of isotonic Ringer's solution at room temperature (control, table 2). It will be observed that the muscles taken from the desiccated frogs (A and B) gained weight continuously for a considerable period of time, and that in each instance before an equilibrium had been established, the amount of water absorbed was essentially equivalent to that which had been lost by desiccation. Other similar experiments in which the frogs had been desiccated to a lesser degree gave essentially the same re-

sults. It is therefore clear that the loss of water in desiccated frogs has been from tissues throughout the body, and has not been a loss merely from the lymph of the subcutaneous lymph sinuses. When we compare the behavior of the desiccated muscles with that of the controls, we observe that the weight of the muscle which had not been desiccated has remained practically stationary for a period of twenty-two hours. The above experiments on desiccated muscles, like those made on the living frog (fig. 1), clearly indicate that when the osmotic pressure of the muscle has been raised above that of the fluid in which the muscles are immersed, the muscles will gain weight by absorbing water and will continue to do so until an equilibrium is established.

Let us now see how we may apply the results obtained by the above experiments to the muscles taken from oedematous frogs, in which an oedema has been experimentally produced by ligating either the leg or the ureters.

We know that lymph in the subcutaneous lymph sinuses gains access to the blood plasma through the lymph hearts and that some of this plasma escapes, as lymph, through the capillary walls of the blood vessels into the tissue spaces, where it becomes available for absorption by the muscles and other tissues. If the osmotic pressure of the lymph in the subcutaneous lymph sinuses of an oedematous ligated frog should, by the gradual addition to the lymph of water through the integument, become lower than that of a resting normal muscle, we should expect that in time the osmotic pressure of the lymph escaping into the tissue spaces would also become lower than that of normal resting muscle, so that, in virtue of differences in osmotic pressure, normal resting muscles would gain weight when immersed in lymph taken from an oedematous frog and oedematous muscles would lose weight when immersed in a Ringer's solution isotonic with normal resting muscle.

On a priori grounds one might expect that when water is constantly and continuously entering the subcutaneous lymph sinuses of a frog in water, and that when all means of egress

of lymph from the ligated area has been discontinued, the concentration of salts in the lymph of the ligated area would gradually become lowered, as would likewise its osmotic pressure. The following experiments show that such has

TABLE 2

Behavior of gastrocnemius muscles from desiccated frogs (A and B) in Ringer's solution approximately isotonic with normal resting muscle.
Control: Behavior of gastrocnemius muscles of normal healthy frog (C) in Ringer's solution approximately isotonic with normal resting muscle.
Ringer's solution: $\triangle = 0.445°$ — pH, 7.0. Room temperature.

EXPERIMENT	HOURS	LEFT GASTROCNEMIUS OF DESICCATED FROG	CONTROL: RIGHT GASTROCNEMIUS OF DESICCATED FROG
		Gram (per cent)	*Gram (per cent)*
668 (A)	0.	0.445 (0)	0.445 (0)
	1.00	0.49 (+10.1)	0.49 (+10.1)
	2.00	0.50 (+12.3)	0.50 (+12.3)
	4.00	0.52 (+16.8)	0.52 (+16.8)
	5.00	0.53 (+19.0)	0.53 (+19.0)
	7.00	0.54 (+21.3)	0.54 (+21.3)
	8.00	0.56 (+26.0)	0.56 (+26.0)
	21.00	0.57 (+28.0)	0.57 (+28.0)
	26.00	0.57 (+28.0)	0.57 (+28.0)
669 (B)	0.	0.26 (0)	0.25 (0)
	1.00	0.29 (+11.5)	0.28 (+12.0)
	2.00	0.30 (+15.3)	0.30 (+20.0)
	3.00	0.31 (+19.2)	0.31 (+24.0)
	4.00	0.31 (+19.2)	0.32 (+28.0)
	5.00	0.31 (+19.2)	0.32 (+28.0)
	6.00	0.31 (+19.2)	0.32 (+28.0)
	7.00	0.32 (+23.0)	0.33 (+32.0)
	8.00	0.32 (+23.0)	0.33 (+32.0)
	10.00	0.33 (+27.0)	0.33 (+32.0)
	12.00	0.33 (+27.0)	0.33 (+32.0)
670 (C)	0.	0.34 (0)	0.34 (0)
Control	1.00	0.34 (0)	0.34 (0)
	2.00	0.35 (+3.0)	0.35 (+3.0)
	3.00	0.35 (+3.0)	0.35 (+3.0)
	4.00	0.35 (+3.0)	0.35 (+3.0)
	5.00	0.35 (+3.0)	0.35 (+3.0)
	7.00	0.35 (+3.0)	0.35 (+3.0)
	10.00	0.35 (+3.0)	0.35 (+3.0)
	12.00	0.35 (+3.0)	0.35 (+3.0)
	22.00	0.35 (+3.0)	0.35 (+3.0)

been found to be the case with lymph taken from the subcutaneous lymph sinuses of the leg of a frog in which an oedema has been experimentally produced as the result of ligation.

The legs of three large bullfrogs (R. catesbiana) were ligated near the thigh, and the frogs were placed in water. At the end of forty-eight hours, 30 cc. of bloody lymph was removed from the subcutaneous lymph sinuses of the legs of the three frogs and 30 cc. of clear straw-colored lymph was obtained from the coelom (ascites) of one of the three.

The $\triangle$ or freezing-point depression of this lymph, as well as its hydrogen-ion concentration, were kindly determined for me by my colleague, Doctor Marshall, of the Department of Physical Chemistry. They were as follows:

Leg lymph, obtained from three frogs, $\triangle = 0.394°$ — pH, 4.97
Coelom lymph, obtained from one frog, $\triangle = 0.368°$ — pH, 7.60

The freezing-point lowerings are correct within 0.005 of a degree.

From the above determinations it is evident that the osmotic pressure of lymph obtained from the oedematous area is lower than that of Ringer's solution ($\triangle = 0.445°$) which has been found to be approximately isotonic with the resting muscle of a normal frog.

The following type experiments tabulated in tables 3 and 4 fully bear out the views previously expressed—that the resting muscle of a normal frog (table 3) should gain weight when immersed in lymph obtained from the subcutaneous lymph sinuses of an oedematous area, and that an oedematous muscle (table 4) should lose weight when immersed in Ringer's solution, approximately isotonic with resting muscle taken from a normal frog.

The type of experiment shown in tables 3 and 4 has been repeatedly made on frogs of different species and with lymph taken at different times from different frogs. We therefore believe it may be stated that the differences in weight of the muscles observed in these experiments result from differences in osmotic pressure which exist in the intermuscular

and intramuscular fluids, and in the fluids in which the muscles are immersed.

In two of the ligated bullfrogs it is interesting to note the presence of an abnormal and excessive accumulation of lymph in the coelom, a portion of the body not shut off by a ligature from the general circulation. Such an accumulation of lymph

TABLE 3

Behavior of gastrocnemius muscles from normal healthy frogs, in lymph from oedematous subcutaneous lymph sinuses of ligated legs of other frogs.
Control: Behavior of gastrocnemius muscles from same frogs, in Ringer's solution approximately isotonic with normal resting muscle.
Ringer's solution: $\Delta = 0.445° - pH$, 7.0. Room temperature.

EXPERIMENT	HOURS	LEFT GASTROCNEMIUS IN LYMPH	CONTROL: RIGHT GASTROCNEMIUS IN RINGER
		Gram (per cent)	*Gram (per cent)*
671	0.	0.20 (0)	0.20 (0)
	1.00	0.21 (+5.0)	0.20 (0)
	2.00	0.21 (+5.0)	0.20 (0)
	6.00	0.22 (+10.0)	0.20 (0)
	7.00	0.23 (+15.0)	0.20 (0)
	12.00	0.23 (+15.0)	0.20 (0)
	24.00	0.24 (+20.0)	0.20 (0)
	25.00	0.24 (+20.0)	0.20 (0)
	31.00	0.25 (+25.0)	0.20 (0)
	32.00	0.25 (+25.0)	0.20 (0)
673	0.	0.29 (0)	0.29 (0)
	1.00	0.30 (+3.4)	0.29 (0)
	2.00	0.31 (+6.9)	0.29 (0)
	6.00	0.32 (+10.3)	0.29 (0)
	7.00	0.32 (+10.3)	0.29 (0)
	23.00	0.30 (+3.4)	0.28 (−3.4)

in the coelom (ascites) indicates that, in addition to the oedema in the leg, an oedematous condition has been established throughout the remaining portion of the body. This implies that more water is entering the subcutaneous lymph sinuses through the integument than can simultaneously be excreted by the kidneys. In these frogs the osmotic pressure of the lymph would necessarily be below what we ordinarily regard as normal.

A marked difference is observed also between the pH value of the lymph obtained from the ligated legs and that found in the coelom. The greater acidity of the lymph from the ligated area (pH, 4.97) is apparently associated with metabolic changes in the muscles, which for a considerable period of time have been completely shut off from the general circulation. The pH value of the coelom lymph was found to be essentially normal (pH, 7.60); the reason for this is, appar-

TABLE 4

Behavior of oedematous gastrocnemius muscle from ligated leg of frog, in Ringer's solution approximately isotonic with normal resting muscle.
Control: Behavior of gastrocnemius muscle from unligated leg of same frog, in Ringer's solution approximately isotonic with normal resting muscle.
Ringer's solution: $\triangle = 0.445° - pH$, 7.0. Room temperature.

EXPERIMENT	HOURS	OEDEMATOUS MUSCLE	CONTROL
		Gram (per cent)	*Gram (per cent)*
654	0.	0.86 (0)	0.72 (0)
	1.00	0.85 (—1.1)	0.72 (0)
	2.00	0.84 (—2.3)	0.72 (0)
	7.00	0.81 (—5.8)	0.72 (0)
	23.00	0.78 (—9.3)	0.72 (0)
	26.00	0.78 (—9.3)	0.72 (0)
656	0.	0.87 (0)	0.71 (0)
	1.00	0.84 (—3.4)	0.71 (0)
	2.00	0.83 (—4.6)	0.71 (0)
	5.00	0.81 (—6.9)	0.71 (0)
	10.00	0.80 (—8.0)	0.71 (0)
	23.00	0.78 (—10.3)	0.70 (—1.4)

ently, that in all parts of the body not shut off by a ligature from the general circulation, waste products are eliminated in the usual way. The fact that lymph taken from one part of an oedematous frog gives an essentially normal reaction, while lymph taken from another part of the same frog gives an acid reaction, is evidence of the fact that acidity does not in all forms of oedema invariably play the essential rôle, as is maintained by Martin Fischer. From the investigations of Loeb ('98) we know that muscles may swell and increase in

weight when immersed in an isotonic saline solution acidulated with lactic acid; but we can nevertheless show by experiment that no increase in weight occurs when normal resting muscle is immersed in an acidulated isotonic Ringer's solution having a pH value of 4.8. As shown in table 5, the weight of such muscles remains practically stationary for a period of twenty-three hours, and in this respect their behavior resembles that of normal resting muscles immersed in isotonic Ringer's solution having a pH value of 7.0. We may therefore conclude that the swelling of muscles, or the

TABLE 5

Behavior of normal resting gastrocnemius muscles from three different frogs (A, B, and C), when muscles are immersed in the isotonic Ringer's solution acidulated with lactic acid.

Isotonic Ringer's solution: $\triangle = 0.445°$ — pH, 4.8. Room temperature.

HOURS	GRAM (PER CENT)		
	A	B	C
0.	0.21 (0)	0.24 (0)	0.34 (0)
1.00	0.21 (0)	0.25 (+4.1)	0.35 (+3.0)
2.00	0.21 (0)	0.25 (+4.1)	0.35 (+3.0)
5.00	0.21 (0)	0.25 (+4.1)	0.35 (+3.0)
8.00	0.21 (0)	0.25 (+4.1)	0.35 (+3.0)
23.00	0.22 (+4.7)	0.25 (+4.1)	0.35 (+3.0)

intracellular (interstitial?) form of oedema observed in ligated oedematous frogs, is not necessarily associated with an overproduction of acid in the tissues, but results rather from differences in osmotic pressure that exist in the tissue fluids, and in the lymph in the tissue spaces from which the cells of the tissues constantly receive their lymph supply.

Under normal conditions, it is through the agency of the vascular system that an excess of free water in the body is made available to the kidneys, and is by them excreted. In the case of an oedema resulting from the ligation of the leg or the ligation of the ureters, this means of eliminating free water from the body is either locally or generally discon-

tinued. The principle involved is purely mechanical. We know that when normal frogs are placed in water at any temperature within the range of that of their natural environment, a balance is soon established in the body between the incoming and outgoing flow of water, so that free water in excess of normal does not accumulate in the body. Furthermore, we know that the rate at which water is transported through the integument into the subcutaneous lymph sinuses increases with the temperature, so that more water enters the sinuses at high than at low temperatures. This has been demonstrated in table 1, in which is shown the behavior of those frogs and toads whose ureters have been ligated when placed in water at different temperatures. If the ureters of these same frogs and toads had not been ligated, any increase in the amount of water entering the body at the higher temperatures would have been almost simultaneously excreted by the kidneys, and a normal balance would thereby have been established between the incoming and outgoing flow of water. This still further emphasizes the fact, maintained also by others, that the kidneys serve chiefly as passive agents in the sense that they do not seek water, but excrete only that amount made available to them through the agency of the vascular system. If at higher temperatures the increased amount of water entering the subcutaneous lymph sinuses through the integument is, through the agency of the vascular system, almost simultaneously excreted by the kidneys, it is evident that the rate at which this free water is supplied to the kidneys is correspondingly accelerated. If this were not the case, free water would gradually accumulate in the body in excess of normal, for the reason that less water would then be able to reach the kidneys than was simultaneously being transported through the integument into the subcutaneous lymph sinuses. The question therefore arises whether it would be possible by gradually raising the temperature of the water to reach a point at which, within a given time, more water actually entered the body than could simultaneously be conveyed to the renal circulation for ex-

cretion by the kidneys. If such a condition could be brought about, it is plain that in course of time water would accumulate in the body in excess of normal.

Experiments of this character made by the writer have shown that when normal frogs are transferred from water at room temperature to water at 36°C., such frogs, after remaining in water at this higher temperature for about thirty minutes, almost invariably show a slight loss in body weight. As a matter of fact, it has been observed by the writer that, within limits of temperature ordinarily consistent with their natural environment, frogs at first usually lose weight when taken from water at a low temperature to water at a higher temperature, and gain weight when taken from water at a high to water at a lower temperature. In the average normal frog the power of the kidneys to maintain at 36°C. a normal balance between the incoming and outgoing flow of water signifies in the kidneys not only increased action, but also a reserved functional capacity to meet quite unusual conditions. While the behavior necessarily differs in different cases, 36°C. is about the average limit at which, in a normal frog, a normal balance can be maintained between the incoming and outgoing flow of water, provided, however, the duration of the experiment is not too extended.

If we may regard 36°C. as about the average limit of temperature at which the kidneys can maintain a normal balance between the incoming and outgoing flow of water, what conditions are met with when frogs are placed in water at still higher temperatures? While it has thus far proved impossible to determine for all cases the exact limits of temperature at which the kidneys cease to meet the normal requirements of excreting water, it has nevertheless been possible to demonstrate that when frogs are placed in water at a temperature somewhere between 36° and 39°C., a slight gain in body weight immediately follows. And, furthermore, this gain in weight, slight though it may be, is within a short time invariably accompanied by coma and cardiac inefficiency. The result is that the rate of flow in the vascular channels is

correspondingly diminished, so that through the agency of the vascular system less water reaches the kidneys than simultaneously enters the body through the integument. Such conditions obtain when frogs are subjected to these high temperatures; since, however, frogs are unable for any extended period to endure them, an actual oedematous condition of the body cannot be experimentally produced in the living frog by thus increasing the temperature of the water.

Under natural conditions of environment, however, oedematous frogs with red-leg disease do continue to live a considerable time after cardiac inefficiency is manifest. The writer will show later that this inefficiency prevents the establishment of the normal balance between the incoming and outgoing flow of water, and so is itself a contributing factor of the oedema. Moreover, dead frogs, when placed in water at any temperature, gain weight continuously through a considerable period of time. This is because though after death water continues to be transported in the usual manner through the integument, this water, owing to a complete cessation of circulation, cannot be transported to the kidneys. These two observations indicate the relation that exists between cardiac inefficiency and oedema.

A brief résumé of the chief observations made in the preceding pages leads us to the following conclusions:

1. Oedema manifests itself in the frog in two forms which we may distinguish as subcutaneous and intracellular (interstitial?).

2. Any factor, either local or general, which may prevent the establishment of a normal balance between the incoming and outgoing flow of water, such as a deficiency in the development of the kidneys, ligation of a limb, ligation of the ureters, or cardiac inefficiency, will serve as a contributing cause of oedema.

3. Subcutaneous oedema is an excessive accumulation of lymph in the subcutaneous lymph sinuses and is invariably accompanied by an intracellular (interstitial?) oedema or swelling of the tissues, which results from differences in os-

motic pressure existing in the tissues and in the lymph which escapes through the capillary walls of the blood vessels into the tissue spaces.

It has occurred to the writer that these same principles are active in the causation of the oedema which accompanies a disease very common among frogs known as *red-leg*. To this we shall now turn our attention.

ON THE OEDEMA WHICH IN FROGS ACCOMPANIES RED-LEG DISEASE

According to Emerson and Norris ('05), red-leg disease, which is widely distributed throughout North America and Europe, is due to the presence in the frog of Bacillus hydrophilus fiscus.[3] They state that the predisposing causes of the disease are lower resistance from heat, anemia, and lesions of the skin, which seems to be the portal of entry of the infection. The symptoms which characterize the disease are congestion of the ventral surface of the body and more or less ulceration in and haemorrhage beneath the skin. In advanced cases of red-leg, the abdominal viscera, and especially the stomach and intestines, may become greatly congested, as may also the muscles of the body in general. As the disease progresses and congestion becomes more general, it may be accompanied by more severe haemorrhages and by degenerative changes in the tissues. The disease is usually fatal, and a gradual failure to respond to stimuli is followed by coma and death. The oedema which accompanies red-leg disease may be either localized or generalized in character. It may be confined to one or to both hind limbs, and either to the entire limb or to the limb above or below the knee-joint. When the oedema is generalized in character, the entire body, including the limbs, may be generally and uniformly swollen.

Figure 2 is a photograph of a frog with red-leg disease, in which a rather intense form of localized subcutaneous oedema of the right thigh has developed. Figure 3 also is a photograph of a frog with red-leg disease, but here there is a local-

[3] Cultures were made by Mr. Vincent Gregg, who identified the bacillus mentioned.

ized swelling of the right leg, and in addition the entire body is generally and uniformly swollen. In the latter, therefore, we have a case in which a localized as well as a generalized subcutaneous oedema has been developed in the same individual—a condition which, as previously mentioned, can be simultaneously produced in the living frog by applying a

Fig. 2 Photograph of a frog with red-leg disease, showing a localized subcutaneous oedema of right thigh.

ligature to the thigh and by ligating the ureters. The presence of a localized and a generalized oedema in the same individual suggests the possibility that, as in the case of the double-ligation experiment, the cause of the localized swelling may be influenced by local rather than by general conditions; otherwise the lymph would be more uniformly distributed throughout the subcutaneous lymph sinuses.

A normal healthy frog, when transferred from water at one temperature to water at another or when left a long period of time in water at one temperature, usually either loses, or remains practically stationary in weight. Should

Fig. 3 Photograph of a frog with red-leg disease, showing a localized subcutaneous oedema of right leg and a generalized subcutaneous oedema of the rest of the body.

it gain weight, the gain is only temporary, and never does the gain continue through an extended period. On the other hand, when frogs with well-defined external symptoms of red-leg disease remain in water at any temperature within the limits of that of their natural environment, they immediately begin

to gain in weight, and continue to do so as long as the disease is progressive. This continuous gain indicates that more water is being transported through the integument into the subcutaneous lymph sinuses than is simultaneously being excreted by the kidneys, and leads to the gradual production of a localized or generalized oedema, subcutaneous as well as intracellular (interstitial?) in character.

In table 6 is shown a number of experiments which bear out the above statement that if frogs with well-defined symptoms of red-leg disease remain in water, they gain weight continuously. As the disease progresses, it will be observed that, in course of time, the frogs become weak and sluggish and react slowly to stimuli. This condition is succeeded by coma and death follows. The disease is usually fatal at room temperature; Emerson and Norris state, however, that at low temperatures its progress may be arrested.

In experiment 161 (table 6) an apparently healthy frog, showing no external symptoms of red-leg, and selected for the purpose of studying its normal behavior, was observed to lose weight continuously during a period of about four hours, after being transferred from water at room temperature to water at 33°C. Up to this point, the behavior of the frog was normal in respect to the balance that had been established between the incoming and outgoing flow of water. After the fourth hour, however, external symptoms of red-leg made their appearance, and after this time the weight of the frog increased continuously.

The selection for experiment of frogs which one would commonly regard as healthy and free from red-leg has proved in some cases a difficult matter. While the character of its desiccation recovery curves (curves *1*, *2*, and *3* in fig. 1) and its behavior when placed in water generally indicate whether a frog is in healthy condition, the unexpected appearance of red-leg in frogs that in these respects give normal reactions is by no means an uncommon occurrence in the experience of the writer. An example of this is well illustrated by curve *6* in figure 1. The frog from which this curve was obtained

had previously been used for a number of experiments extending over a considerable period of time. It had therefore become extremely thin and weak at the time of this last experiment. After injecting into the subcutaneous lymph sinuses 1 cc. of a 0.975 per cent solution of NaCl, the writer put the frog into tap-water at 26°C. Curve *6* in figure 1 shows us that, after a slight gain which was followed by a considerable loss in weight, the frog began to gain weight continuously. This gain was accompanied by red-leg symptoms which unexpectedly made their appearance some time between the eighth and twelfth hours of the experiment.

Having considered the general symptoms of red-leg disease in frogs and the general behavior in water of frogs so afflicted, let us now attempt to explain the presence of the oedema, both localized and generalized, associated with this disease, on the basis of those factors involved in the production of oedema which we have outlined in the preceding pages.

We may state that the presence in frogs of a localized oedema indicates the probability, at least, that the oedema of this character is not due to inefficiency in kidney function. As a matter of fact, an examination of the urine of frogs in which the oedema is localized has shown that the oedema is not always accompanied by albuminuria. Albuminuria is of frequent occurrence in red-leg disease, however, when congestion has become general.

If, by the aid of a binocular microscope, we follow the progress of congestion in a case of red-leg in which at first the web of the foot is alone congested, we meet with conditions which we have the right to assume represent a typical clinical picture of what may similarly occur elsewhere in the body when congestion has become more general. The condition which we actually observe in the foot is the establishment of a localized mechanical block in the vascular system, predisposing to the establishment of a typical case of localized subcutaneous oedema of exactly the same character as that which would have been formed had the foot of the frog been ligated. If we follow the progress of the disease as it spreads

TABLE 6

Showing behavior of living frogs with red-leg disease, when frogs are placed in finger-bowls containing 100 cc. of tap-water

EXPERIMENT	WEIGHT OF FROG	HOURS IN WATER	TEMPERATURE OF WATER (°C.)
	Gram (per cent)		
597	55.95 (0)	0.	15
	56.85 (+1.6)	1.00	
	58.98 (+5.4)	46.00	
	59.15 (+5.7)	52.00	30
	59.63 (+6.5)	53.00	15
	61.35 (+9.6)	57.00	
	Frog, sluggish.		
	63.55 (+13.5)	69.00	
	Dead.		
598	52.53 (0)	0.	23
	53.99 (+2.7)	6.30	
	57.55 (+9.5)	24.00	
	Frog, comatose.		
	58.00 (+10.4)	30.00	
	Dead.		
600	25.08 (0)	0.	23
	27.44 (+9.4)	23.00	
	Frog, sluggish. Killed.		
641	36.25 (0)	0.	21
	39.07 (+7.7)	22.30	
	40.32 (+11.2)	31.00	
	42.55 (+17.3)	45.00	
	Frog, sluggish		
642	22.97 (0)	0.	21
	23.48 (+2.2)	22.00	
	25.55 (+11.2)	33.00	
	27.45 (+19.5)	45.00	
	Frog, comatose. Killed.		
643	18.95 (0)	0.	21
	19.8 (+4.4)	11.00	
	20.20 (+6.5)	24.00	
	22.67 (+19.6)	47.00	
	Frog, comatose. Killed.		
644	33.37 (0)	0.	21
	35.24 (+5.6)	8.45	
	Frog, comatose. Killed.		
645	19.67 (0)	0.	21
	21.7 (+10.3)	23.00	
	Frog, active. Killed.		

TABLE 6—*Continued*

EXPERIMENT	WEIGHT OF FROG	HOURS IN WATER	TEMPERATURE OF WATER (°C.)
	Gram (per cent)		
104	41.25 (0)	0.	11
	42.4 (+2.7)	5.00	
	43.3 (+4.9)	17.00	
	43.81 (+6.2)	23.30	
	45.05 (+9.2)	28.30	
	47.82 (+15.9)	41.00	
	48.43 (+17.4)	44.00	
	Frog, sluggish.		
161	31.42 (0)	0.	33
	No external symptoms of red-leg.		
	30.85 (—1.8)	1.00	
	30.6 (—2.6)	3.40	
	31.87 (+1.4)	5.00	
	External symptoms of red-leg.		
	32.9 (+4.7)	6.00	
	34.24 (+8.9)	18.50	
	Frog, active.		
585	25.58 (0)	0.	21
	26.9 (+5.1)	3.15	
	32.58 (+27.3)	23.00	
	Frog, sluggish. Killed.		

from the foot to the leg, we observe that soon the latter becomes swollen. When, in addition to the foot and leg, the abdominal region becomes affected, a similar swelling of this region becomes evident, and thus the entire body presents the appearance of being swollen generally. The variable manner in which localized oedema manifests itself is essentially indicative of the variable manner in which local conditions may arise, predisposing to its establishment.

Frogs usually remain active and respond to stimuli when the oedema is purely localized in character, as when one or both legs are affected. As soon, however, as congestion becomes more general, and especially when the gastro-intestinal tract is involved, the frogs become weak and sluggish, and soon coma follows. At this time, owing to cardiac inefficiency, the oedema becomes general throughout the body. The ex-

planation of this is that at this stage more free water is generally being added to the lymph in the subcutaneous lymph sinuses than can simultaneously be transported to the kidneys through the agency of the heart and systemic blood vessels. Lymph therefore gradually accumulates in the subcutaneous lymph sinuses of the body, while formerly its accumulation was regulated and controlled by local conditions. As stated above, the oedematous condition of the body which results when dead frogs remain in water is owing to the fact that, while water still continues to be transported through the integument into the subcutaneous lymph sinuses, all means of transportation to the kidneys by the vascular system has been discontinued. In advanced stages of red-leg disease, cardiac inefficiency similarly acts as a leading factor in rendering it impossible for as much free water to reach the kidneys and to be excreted by them as simultaneously enters the subcutaneous lymph sinuses through the integument, so that lymph generally accumulates in the body in excess of what experiment has shown us to be normal.

Up to this point we have considered certain causes which may account for the occurrence of localized and generalized oedema commonly associated with red-leg disease. We have found also that in frogs whose legs or ureters have been ligated, the resulting subcutaneous oedema is accompanied by an intracellular (interstitial?) oedema; the same is the case in red-leg disease. We, therefore, come now to an analysis of the behavior of muscles from frogs with red-leg disease, under experimental conditions similar to those used in our study of muscles from frogs whose legs or ureters had been ligated.

Should oedema of muscles in red-leg disease be due to an osmotic reaction, we should predict that muscles of a normal healthy frog would gain in weight when immersed in lymph obtained from a frog with red-leg disease; and, conversely, that the muscles of a frog with red-leg disease would lose in weight when immersed in a Ringer's solution, isotonic with normal resting muscle. The reason for this would be that

such behavior would result if the osmotic pressure of lymph and of muscle in red-leg disease were lower than that of normal resting muscle. That these predictions have been fulfilled is shown by the following experiments.

In table 7 are shown experiments in which the left gastrocnemius muscles of three normal healthy frogs were im-

TABLE 7

Behavior of gastrocnemius muscles from normal healthy frogs, in lymph from subcutaneous lymph sinuses of frogs with red-leg disease.

Controls: Behavior of gastrocnemius muscles of same frogs, in Ringer's solution approximately isotonic with normal resting muscle.

Ringer's solution: $\Delta = 0.445°$ — pH, 7.0. Room temperature.

EXPERIMENT	HOURS	LEFT GASTROCNEMIUS IN LYMPH	CONTROL: RIGHT GASTROCNEMIUS IN RINGER
		Gram (per cent)	*Gram (per cent)*
657	0.	0.48 (0)	0.49 (0)
	1.00	0.51 (+6.2)	0.49 (0)
	2.00	0.52 (+8.3)	0.49 (0)
	7.00	0.53 (+10.4)	0.49 (0)
	23.00	0.53 (+10.4)	0.49 (0)
660	0.	0.45 (0)	0.45 (0)
	1.00	0.48 (+6.6)	0.45 (0)
	2.00	0.49 (+8.8)	0.45 (0)
	7.00	0.51 (+13.3)	0.45 (0)
	23.00	0.52 (+15.5)	0.45 (0)
	26.00	0.52 (+15.5)	0.45 (0)
681	0.	0.29 (0)	0.29 (0)
	1.00	0.30 (+3.4)	0.29 (0)
	4.00	0.31 (+7.0)	0.29 (0)
	8.00	0.32 (+10.3)	0.29 (0)

mersed in lymph from frogs with red-leg disease, and the right gastrocnemius muscles (control) of the same frogs were immersed in Ringer's solution approximately isotonic with normal resting muscle. In each instance the muscle immersed in lymph gained weight while the weight of the muscle in isotonic Ringer's solution remained stationary. Also, as shown by the controls in tables 8, 9, and 10, it will be observed that muscles from frogs with red-leg disease lost

weight when immersed in Ringer's solution approximately isotonic with normal resting muscle. As stated above, this behavior would be expected, provided that in red-leg disease the osmotic pressure of the lymph were lower than that of normal resting muscle. An examination of the lymph has shown such to be the case.

The $\triangle$ or freezing-point depression and hydrogen-ion concentration of lymph obtained from frogs (Rana catesbiana) with red-leg disease have been determined by my colleague, Doctor Marshall, to be as follows:

Frog A, $\triangle = 0.330° \pm 0.010°$C.
pH = 7.5
Frog B, $\triangle = 0.29° \pm 0.010°$C.
pH = 8.1

The value of $\triangle$ in frog B is for a partially coagulated lymph, and may differ from the original value for the same, owing to the absorption of electrolytes from the solution in the process of coagulation.

A number of other experiments have been made which still further bear out the view that the oedema which accompanies red-leg disease can be explained on the grounds that muscles take up and hold water as long as the osmotic pressure of the lymph supplied to the muscles continues to remain lower than that of the muscles.

In table 8 two experiments are shown in which the gastrocnemius muscle of a frog with red-leg disease was immersed in lymph obtained from the subcutaneous sinuses of the ligated leg of a normal frog. In one instance (experiment 654) the osmotic pressure of the muscle was higher than that of the lymph, and the muscle gained 13.9 per cent in weight before an equilibrium was established. In the other experiment (experiment 659) the lymph was essentially isotonic with the red-leg muscle, and the weight of the latter remained practically stationary. The behavior of the controls—red-leg muscles immersed in Ringer's solution approximately isotonic with normal resting muscle—was typical of that of all such muscles when observed under similar experimental

conditions. These muscles lost weight when immersed in Ringer's solution approximately isotonic with normal resting muscle.

In table 9 are shown experiments in which the gastrocnemius muscles of a frog with red-leg disease were immersed in lymph obtained from the same frog. In one experiment (experiment 661) the lymph was taken from the subcutaneous lymph sinuses, and in the others from the coelom. It will be

TABLE 8

Behavior of gastrocnemius muscles from frogs with red-leg disease, in lymph from subcutaneous sinuses of ligated legs of normal healthy frogs.
Controls: Behavior of gastrocnemius muscles from same red-leg frogs, in Ringer's solution approximately isotonic with normal resting muscle.
Ringer's solution: $\triangle = 0.445° - pH$, 7.0. Room temperature.

EXPERIMENT	HOURS	LEFT GASTROCNEMIUS IN LYMPH	CONTROL: RIGHT GASTROCNEMIUS IN RINGER
		Gram (per cent)	*Gram (per cent)*
654	0.	0.43 (0)	0.45 (0)
	1.00	0.45 (+4.6)	0.44 (—2.2)
	2.00	0.45 (+4.6)	0.44 (—2.2)
	7.00	0.48 (+11.6)	0.39 (—13.3)
	23.00	0.49 (+13.9)	0.35 (—22.2)
	26.00	0.49 (+13.9)	0.35 (—22.2)
659	0.	0.35 (0)	0.34 (0)
	1.00	0.35 (0)	0.33 (—2.9)
	2.00	0.35 (0)	0.33 (—2.9)
	7.00	0.34 (—2.8)	0.31 (—8.8)
	24.00	0.34 (—2.8)	0.30 (—11.7)
	26.00	0.34 (—2.8)	0.30 (—11.7)

observed that the muscles when first placed in lymph either gained slightly or remained stationary in weight, and that the controls lost weight when placed in Ringer's solution approximately isotonic with normal resting muscle.

Jacques Loeb ('98) has stated that when a frog's muscle is immersed in a 0.7 per cent solution of NaCl, to which a trace of acid has been added, the muscle takes up and holds water. This phenomenon forms the central idea of the colloid-chemical theory of Martin Fischer; hence it is inter-

esting to observe the effects produced on muscles obtained from oedematous red-leg frogs, when immersed in an acidulated Ringer's solution approximately isotonic with normal resting muscle.

TABLE 9

Behavior of gastrocnemius muscles from frogs with red-leg disease, in lymph obtained from same frogs.

Controls: Behavior of gastrocnemius muscles from same frogs, in Ringer's solution approximately isotonic with normal resting muscle.

Ringer's solution: $\Delta = 0.445°$ — pH, 7.0. Room temperature.

EXPERIMENT	HOURS	LEFT GASTROCNEMIUS IN LYMPH (LEG SINUS)	CONTROL: RIGHT GASTROCNEMIUS IN RINGER
		Gram (per cent)	*Gram (per cent)*
661	0.	0.50 (0)	0.49 (0)
	1.00	0.51 (+2.0)	0.48 (—2.0)
	2.00	0.51 (+2.0)	0.47 (—4.0)
	6.00	0.52 (+4.0)	0.43 (—12.2)
	23.00	0.52 (+4.0)	0.41 (—16.3)
	25.00	0.50 (0)	0.41 (—16.3)
		Lymph (coelom)	
682	0.	0.62 (0)	0.60 (0)
	1.00	0.62 (0)	0.59 (—1.6)
	2.00	0.62 (0)	0.58 (—3.3)
	24.00	0.62 (0)	0.55 (—8.3)
	26.00	0.62 (0)	0.55 (—8.3)
		Lymph (coelom)	
685	0.	0.31 (0)	0.31 (0)
	1.00	0.31 (0)	0.30 (—3.2)
	4.00	0.31 (0)	0.29 (—6.4)
	9.00	0.29 (—6.4)	0.28 (—9.6)
	21.00	0.27 (—12.9)	0.28 (—9.6)

Four experiments of this character are shown in table 10. In two of the experiments, the pH value of the Ringer's solution was 6.5 and in the remaining two it was 4.8. In each experiment the muscles were taken from the same frog. The writer found that the behavior of the muscles in acidulated Ringer was the same as that of the controls which were im-

mersed in Ringer's solution approximately isotonic with normal resting muscle, but having a pH value of 7.0. While we may recognize the fact that under certain conditions acidity plays a definite rôle in causing muscles to take up water, it is

TABLE 10

Behavior of gastrocnemius muscles from frogs with red-leg disease, in Ringer's solution approximately isotonic with normal resting muscle and acidulated with lactic acid.

Controls: Behavior of gastrocnemius muscles from same frogs, in Ringer's solution approximately isotonic with normal resting muscle. $\Delta = 0.445°$. *Room temperature.*

EXPERIMENT	HOURS	LEFT GASTROCNEMIUS IN RINGER (pH, 6.5)	CONTROL: RIGHT GASTROCNEMIUS IN RINGER (pH, 7.0)
		Gram (per cent)	*Gram (per cent)*
662	0.	0.52 (0)	0.495 (0)
	1.00	0.48 (—7.6)	0.46 (—7.0)
	4.00	0.42 (—19.2)	0.40 (—19.1)
	22.00	0.37 (—28.8)	0.37 (—25.2)
	46.00	0.35 (—32.6)	0.35 (—29.3)
663	0.	0.43 (0)	0.44 (0)
	1.00	0.42 (—2.3)	0.44 (0)
	4.00	0.40 (—7.0)	0.43 (—2.2)
	21.00	0.38 (—11.6)	0.39 (—11.3)
	22.00	0.38 (—11.6)	0.39 (—11.3)
	46.00	0.35 (—18.6)	0.36 (—18.1)
		RINGER (pH, 4.8)	
665	0.	0.57 (0)	0.58 (0)
	1.00	0.55 (—3.5)	0.55 (—5.1)
	4.00	0.49 (—14.0)	0.48 (—17.2)
	23.00	0.42 (—26.3)	0.42 (—27.5)
666	0.	0.39 (0)	0.39 (0)
	1.00	0.36 (—7.7)	0.38 (—2.5)
	4.00	0.32 (—17.9)	0.34 (—12.8)
	23.00	0.30 (—23.0)	0.31 (—20.5)

evident that, within the limits indicated, its presence has no material effect of this sort on the oedematous muscles from red-leg frogs. It has already been pointed out by Henderson ('18), Crozier ('18), and others that, on account of the limited hydrogen-ion concentration in the body, the colloid-chemical theory of Fischer is untenable.

Owing to a possible inefficiency in kidney function, due to the presence of albumin in the urine, albuminuria has been regarded as a cause of oedema. In the frog it can be demonstrated by experiment that an oedema does result both when there is a deficiency in development of the renal tubules (McClure, '19) and when the kidneys of a larval frog have been extirpated (Howland, '16, and Swingle, '19). Of this there can be no question. The point at issue at present, however,

TABLE 11

Behavior of a frog, not afflicted with red-leg disease but in which an acute nephritis has been experimentally developed, when placed in tap-water at 29° and 16°C.

HOURS IN WATER	WEIGHT OF FROG	TEMPERATURE OF WATER
	Gram	
0.	27.95	29°C.
	Frog sluggish, albumin present, casts in abundance.	
1.25	27.8	
4.00	26.5	
	Frog sluggish, albumin present, casts still abundant.	
0.	26.5	16°C.
1.00	27.5	
	Frog active, albumin and casts present.	
2.00	27.45	
13.00	26.65	
	Frog active, trace of albumin, no casts observed.	
15.40	26.8	
	Frog active, trace of albumin.	

is whether in red-leg disease the presence of albuminuria causes an actual inefficiency in kidney function sufficient to account in this disease for the gradual and excessive accumulation of lymph in the body (table 6). Frogs with red-leg disease are much less resistant than normal frogs and when placed in water at temperatures beyond the range of their natural environment, almost immediately fall into a comatose condition which is accompanied by cardiac inefficiency. Since cardiac inefficiency is in itself a cause of oedema, one is unable

in these cases to determine in red-leg disease the effect of albuminuria on the efficiency of the kidneys.

In order to test the efficiency of the kidneys in frogs which are not afflicted with red-leg disease, but in which an acute nephritis had been developed, the following procedure was adopted:

Normal frogs were transferred alternately to and fro from water at room temperature to water at 38°C., until an acute nephritis had been developed with albumin and abundant casts showing in the urine. The nephritis apparently resulted from an overfunctioning of the kidneys. This procedure often required several days, and in order not to kill the frog during the experiment, the writer immediately revived a frog as soon as it appeared sluggish or slightly comatose by placing it in water at a much lower temperature. In table 11 we have the behavior of such a frog, which was placed first in water at 29° and later in water at 16°C. During the twenty hours that the frog was in water at 29° and 16°C., there was no gain in weight of any significance; obviously, the albuminuria did not cause any inefficiency in kidney functioning, as a normal balance was maintained in the body between the incoming and outgoing water.[4]

SUMMARY

Oedema, both localized and generalized, manifests itself in red-leg disease in two forms which we may designate as subcutaneous and intracellular (interstitial?) oedema.

A localized and excessive accumulation of lymph in the subcutaneous lymph sinuses results from a local block in the efferent drainage channels. As water is being constantly driven by osmosis through the integument into the subcutaneous lymph sinuses, lymph gradually accumulates in the latter, distal to the block and locally in the sinuses. The osmotic pressure of this oedematous lymph is lower than that of normal resting muscle.

[4] I wish to express my thanks and appreciation to my colleague, Prof. E. Newton Harvey, for the many favors extended to me during the course of this investigation; my thanks are also due Mr. W. P. Agnew for his kindness in preparing the photographs for me.

A generalized and excessive accumulation of lymph in the subcutaneous lymph sinuses is associated chiefly with cardiac inefficiency, as the result of which, when the frog remains in water, more free water continuously enters the subcutaneous lymph sinuses through the integument than can simultaneously be transported to the kidneys through the agency of the vascular system. Hence, lymph gradually accumulates in the subcutaneous lymph sinuses in excess of normal and causes a general distention of the sinuses. The osmotic pressure of this lymph is lower than that of normal resting muscle.

A swelling of tissues accompanies the subcutaneous oedema. This is brought about by differences in osmotic pressure which exist between the fluid within and that supplied to the tissues.

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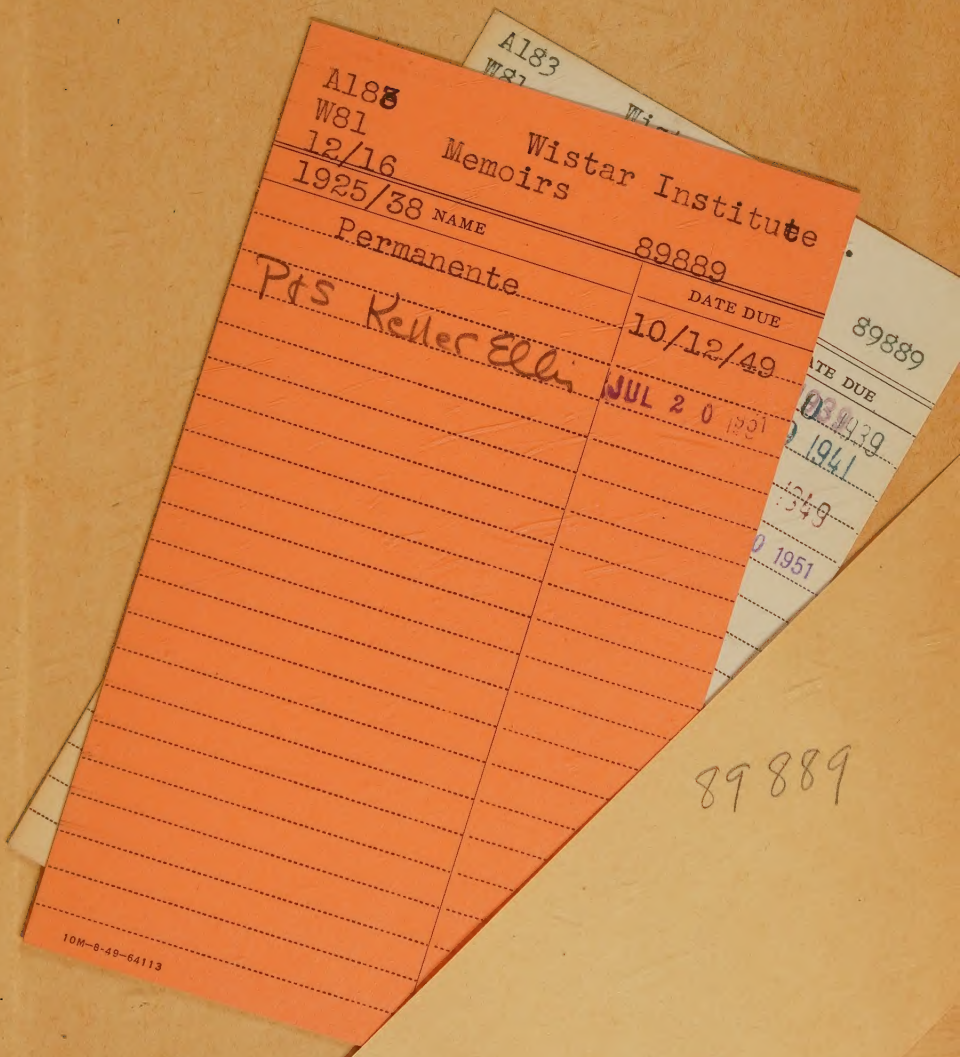
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